

**TRANSMISSION DYNAMICS OF *TOXOPLASMA GONDII*
IN TERRESTRIAL ECOSYSTEMS OF THE CANADIAN WESTERN ARCTIC**

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By

Émilie Bouchard

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Western College of Veterinary Medicine

52 Campus Drive

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ABSTRACT

Toxoplasma gondii is a single-celled parasite infecting a wide range of birds and mammals worldwide. In these warm-blooded animals, it usually causes no symptoms but can cause neurological, ocular, and reproductive problems, especially if the immune system is compromised or if a mammal becomes infected while pregnant. Seroprevalence in some Inuit communities is much higher than in other parts of North America. Inuit are thought to be exposed through handling and consumption of Arctic wildlife. As *T. gondii* are known to produce oocysts in the intestines of felids, and wild and domestic felids are rare in the tundra regions of the Arctic, there are other transmission mechanisms occurring. Previous work has demonstrated that migratory birds are a potential source for introduction of toxoplasmosis, and that Arctic foxes are likely infected through carnivory.

The aim of this research was, first, to determine major routes of transmission of *T. gondii* in a terrestrial Arctic ecosystem at Karrak Lake in the Queen Maud Gulf Bird Sanctuary, Nunavut, in Arctic foxes and Arctic-nesting geese. We also compared molecular and serological techniques used to detect and characterize *T. gondii*. Finally, reindeer were experimentally infected to determine effects and tissue distribution of the parasite. This work will address important food safety and public health aspects by looking for *T. gondii* in two key species, geese and caribou, which are important food sources in northern communities. Caribou populations are declining throughout the Arctic. Knowing what effects this parasite can have on caribou gives us information on whether it causes health problems or not. As for Arctic foxes, looking into seroprevalence and mode of transmission will help us understand how this parasite persists in this particular ecosystem.

We hypothesize that *T. gondii* is maintained via vertical transmission (i.e., female foxes to the pups), in addition to transmission by carnivory. To test this hypothesis, and to determine if any changes in serostatus occurred throughout multiple years, we collected and tested blood samples from live-trapped adult and juvenile Arctic foxes. Samples were analyzed in laboratories by serological methods (Indirect Fluorescent Antibody Test (IFAT), Modified Agglutination Test (MAT)) developed and improved

previously. We also tested wild goose samples serologically, using filter papers, thoracic fluids and fluids from frozen hearts, and via 2 different methods of DNA extraction followed by PCR for a 529 bp repeating segment. The magnetic capture technique allowed use of up to 100g of tissues compared to ≤ 25 mg with traditional DNA extraction methods, thus increasing the chance to find true positives in heart and brain samples. Finally, we investigated the pathology and tissue distribution of *T. gondii* in experimentally infected reindeer to determine which tissues pose the greatest risk for human exposure.

Our work suggests that *T. gondii* is present in newborn Arctic foxes, supporting the hypothesis of vertical transmission. We also proposed that subsequent litters can be infected congenitally, not just litters of females infected for the first time in pregnancy, which are pregnant females without *T. gondii* antibodies at the moment of infection. This can have important implications regarding reproductive success. Changes in serostatus in adult Arctic foxes throughout the years were also noticed, with a higher rate of exposure and seroconversion in mature foxes, as well as a shift of serostatus from positive to negative in an older fox. These findings give us a better understanding of how the parasite can be transmitted and maintained in a terrestrial Arctic ecosystem. Magnetic capture succeeded in detecting higher levels of *T. gondii* in reindeer tissues than traditional DNA extraction, suggesting the use of this technique when large amount of tissues are available. *Toxoplasma gondii* was not detected in wild goose tissues (i.e., brain, heart) following conventional PCR and sequencing, possibly due to low infection rates in Ross's and Lesser Snow Geese, and low sensitivity of the conventional PCR. Antibodies against *T. gondii* were found serologically in both goose species using body fluids, but not following a chloroform clean-up centrifugation on the samples used to remove fat particles. The quality of samples at the time of serological testing as well as the type of samples (body fluids instead of serum) could explain the negative results after using the chloroform centrifugation. We successfully performed MAT for the first time in the field, suggesting that the use of this technique in remote field areas and northern communities is feasible. Finally, *T. gondii* was successfully detected in commonly consumed muscles of experimentally infected reindeer. This result gives us important insight on a potential mechanism for food-borne transmission of

T. gondii in northern communities and raises concern regarding the safety of caribou meat in naturally infected animals. Subtle behavioral changes were observed in reindeer after infection, as well as respiratory problems in one animal.

Numerous studies have demonstrated that *T. gondii* is capable of vertical transmission in a variety of hosts, including humans. High prevalences of the parasite in host populations that are geographically isolated from definitive felid hosts, as is the case in arctic fox populations, are intriguing scenarios to explore the importance of vertical transmission. It may also help to explain the extent of genetic diversity and the relative roles of sexual recombination (oocysts) vs clonal propagation (asexual reproduction). Moreover, using seroprevalence studies, Arctic foxes can be used as sentinels for *T. gondii* in this specific ecosystem. This research will provide information about how Arctic peoples become exposed through important food sources (i.e., geese and caribou) and the health effects of toxoplasmosis in threatened wildlife. We need more information on the significance of food-borne routes of transmission of *T. gondii* in the North in order to implement culturally appropriate and effective local prevention measures.

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DEDICATION

To my family,

and to the North.

TABLE OF CONTENTS

PERMISSION TO USE	i
ABSTRACT.....	ii
ACKNOWLEDGMENTS	v
DEDICATION.....	vii
TABLE OF CONTENTS	viii
LIST OF FIGURES	xi
LIST OF TABLES	xii
ABBREVIATIONS	xiii
CHAPTER 1: General Introduction and Literature Review.....	1
1.1 INTRODUCTION	1
1.2 THESIS OBJECTIVES.....	2
1.3 STUDY AREA	3
1.3.1 Study species within Karrak Lake ecosystem	3
1.4 LITERATURE REVIEW	4
1.4.1 Life cycle of <i>Toxoplasma gondii</i>	4
1.4.2 Transmission	6
1.4.3 Toxoplasma as a zoonotic and veterinary pathogen	7
1.4.4 Geographic Distribution.....	8
1.4.4.1 In Humans	8
1.4.4.2 In the Environment and Wildlife.....	10
1.4.4.3 In the Arctic.....	12
CHAPTER 2: <i>Toxoplasma gondii</i> in Arctic foxes.....	16
2.1 INTRODUCTION	18
2.2 OBJECTIVES	20
2.3 METHODS	20
2.3.1 Study area	20
2.3.2 Fox trapping and blood sampling.....	21
2.3.3 Serological methods	23
2.3.3.1 Modified Agglutination Test (MAT)	23
2.3.3.2 Indirect Fluorescent Antibody Test (IFAT)	23
2.3.4 Aging method	23

2.3.5	Data analysis	24
2.4	RESULTS	25
2.5	DISCUSSION	29
CHAPTER 3: <i>Toxoplasma gondii</i> in Arctic-nesting geese		36
Transition statement		36
3.1	INTRODUCTION	39
3.2	OBJECTIVES	42
3.3	METHODS	42
3.3.1	Study area	42
3.3.2	Field sample collection	43
3.3.3	Filter Paper Elution	43
3.3.4	Serological Analysis: MAT in the field and in laboratory	44
3.3.5	Chloroform extraction method	45
3.3.6	Magnetic Capture	45
3.3.7	Traditional DNA extraction	47
3.3.8	PCR Amplification	48
3.3.8.1	Post Amplification Analysis	48
3.3.9	Data Analysis	49
3.4	RESULTS	49
3.5	DISCUSSION	52
CHAPTER 4: Experimental infection of reindeer		57
Transition Statement		57
4.1	INTRODUCTION	60
4.2	OBJECTIVES	63
4.3	METHODS	63
4.3.1	<i>T. gondii</i> oocysts	63
4.3.2	Animals	63
4.3.3	Reindeer anesthesia protocol	64
4.3.4	Experimental design	64
4.3.5	Euthanasia and necropsies	65
4.3.6	Histology and Immunohistochemistry on reindeer tissues	65
4.3.7	Serology	66
4.3.8	Traditional DNA extraction (commercial kit)	66

4.3.9	Magnetic capture DNA extraction.....	66
4.3.10	PCR Amplification.....	67
4.3.11	Post Amplification Analysis	68
4.3.12	Data analysis.....	68
4.4	RESULTS	69
4.5	DISCUSSION	74
CHAPTER 5: General Discussion and Conclusion		79
5.1	Transmission and diversity of <i>T. gondii</i> in a terrestrial Arctic ecosystem.....	80
5.2	Comparison of serological and molecular techniques: the use of multiple tests, new perspective for remote studies, and magnetic capture DNA extraction maximizes detection probability	84
5.3	Experimentally infected reindeer demonstrated a wide distribution of <i>T. gondii</i> in various tissues with minor clinical effects	87
5.4	Conclusion	91

LIST OF FIGURES

Figure 1 Location of the Karrak Lake goose colony in Nunavut, Canada. Square represents trapping area within the colony and dots are Arctic fox dens with trapping success in 2014-2015. Bigger dot represents the field camp.....	21
Figure 2 MC trial on heart/brain of geese given three different tachyzoite concentrations at four replicates.....	51
Figure 3 Histological section of reindeer (no. 1) muscle containing a <i>T. gondii</i> cyst visualised at 40X after HE (on the left) and immunohistochemical stain (on the right).....	72
Figure 4 Histological section of reindeer (no. 3) diaphragm containing a <i>T. gondii</i> cyst visualised at 40X after HE (on the left) and at 60X after immunohistochemical stain (on the right).....	72
Figure 5 Picture of <i>T. gondii</i> tissue cyst of reindeer (no. 2) brain visualized on compound microscopy (60X).....	72
Figure 6 Histological section after HE of reindeer (no. 2) pleura at 10X (top left) and 40X (top right), of septa at 10X (middle left) and 40X (middle right), and of alveoli at 10X (bottom left) and 40X (bottom right). Focal to coalescing areas of chronic changes are noted, with fibrinous material, eosinophils and histiocytes.....	73

LIST OF TABLES

Table 1.1 Serological prevalence of <i>T. gondii</i> antibodies in adult Arctic foxes by MAT and IFAT for 2014-2015.....	26
Table 1.2 Serological prevalence of <i>T. gondii</i> antibodies in juvenile Arctic foxes by MAT and IFAT for 2014-2015.....	26
Table 2 Long term trends in seroprevalence for <i>T. gondii</i> antibodies in individual, marked adult Arctic foxes at Karrak Lake.....	27
Table 3 Seroprevalence of <i>T. gondii</i> in parents vs litter using IFAT and MAT for summer 2014 and 2015.....	28
Table 4 Goose trial using heart/brain of 3 individuals pooled together for magnetic capture.....	47
Table 5 MAT and results on goose samples collected at Karrak Lake in 2015.....	50
Table 6 Experimental infection design on reindeer.....	68
Table 7 Detection of <i>T. gondii</i> DNA in different organs comparing two DNA extraction methods.....	71

ABBREVIATIONS

CI	Confidence interval
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-linked Immunosorbent Assay
FITC	Fluorescein isothiocyanate
HE	Hematoxylin and Eosin
IFAT	Indirect Fluorescent Antibody Test
IHC	Immunohistochemistry
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
MAT	Modified Agglutination Test
MC	Magnetic capture
PBS	Phosphate-buffered saline
PCR	Polymerase Chain Reaction
QMGMBs	Queen Maud Gulf Migratory Bird Sanctuary
ROGO	Ross's Goose
SNGO	Snow Goose

CHAPTER 1: General Introduction and Literature Review

1.1 INTRODUCTION

Toxoplasma gondii is one of the most successful parasites in the world. Its distribution is worldwide and infects virtually all warm-blooded animals, including wildlife, domestic animals and humans (Tenter et al., 2000; Dubey, 2009d). It is thus a parasite of importance for both wildlife and human health. Serological studies estimate that a third of the world human population has been exposed to, and may be chronically infected with *T. gondii* (Pappas et al., 2009; Weiss and Dubey, 2009). Despite the high prevalence in many mammalian species, including humans, the infection is usually asymptomatic, but may cause neurological, ocular or reproductive problems, especially if the immune system is compromised or if individuals become infected for the first time while pregnant leading to spontaneous abortion or congenital defects in the foetus (Desmonts and Couvreur, 1974).

Toxoplasmosis is particularly important in the Canadian North. Since its definitive hosts of the family Felidae are largely absent above the treeline, the transmission cycle of *T. gondii* remains enigmatic in this specific environment. In the Arctic, the majority of terrestrial and marine mammal species examined, as well as some avian species, have antibodies to *T. gondii* (Dubey, 2009), with seroprevalences ranging from 10-60% in carnivores and 1-40% in herbivores (Jenkins et al., 2013). Moreover, the seroprevalence in some Inuit communities in the Canadian Arctic is much higher than in other parts of the world. The overall prevalence of exposure in Inuit communities in northern Québec is around 60%, which is significantly higher than the North American average of 10-30%, and almost twice the global average of 33% (Tenter et al., 2000; Jones et al., 2007; Robert-Gangneux and Darde, 2012). Food-borne transmission is likely a significant route of infection among northern residents, given their close contact with the land and wildlife, and particularly due to dietary preferences for raw, fermented or dried meat (Messier et al., 2009; Jenkins et al., 2013). Despite the importance of toxoplasmosis in the Canadian

Arctic, the life cycle of *T. gondii* is unclear in Arctic regions, as is the impact on wildlife health and northern food security. It is critical to investigate transmission mechanisms of this parasite in an Arctic ecosystem to give us a better understanding of how *T. gondii* is introduced and maintained, how to prevent transmission to humans, and how to prevent or mitigate the effects on wildlife.

1.2 THESIS OBJECTIVES

- 1) To better understand transmission and diversity of *T. gondii* in a terrestrial Arctic ecosystem (at Karrak Lake in the Queen Maud Gulf Bird Sanctuary, Nunavut).
 - a. To investigate the role of vertical transmission of *T. gondii* in Arctic foxes (i.e., female foxes to pups).
 - b. To explore interannual dynamics of *T. gondii* seroprevalence in Arctic foxes.
 - c. To identify genotype(s) of *T. gondii* present in migratory geese and Arctic foxes at Karrak Lake.
- 2) To compare molecular and serological techniques used to detect and characterize *T. gondii* in Arctic wildlife.
 - a. To compare serological techniques (Modified Agglutination Test vs Indirect Fluorescent Antibody Test) in the laboratory for Arctic fox blood samples.
 - b. To compare molecular and serological methods in wild Arctic-nesting geese.
 - c. To compare a serological method (Modified Agglutination Test) in the field versus laboratory for wild Arctic-nesting geese using body fluids.
 - d. To compare traditional vs magnetic capture PCR for detection of DNA of *T. gondii* in tissues of wild geese and experimentally infected reindeer (*Rangifer tarandus*).
- 3) To determine effects and tissue distribution of *T. gondii* in experimentally infected reindeer.
 - a. To determine clinical effects and pathology of *T. gondii* in experimentally infected reindeer.
 - b. To determine tissue predilection in experimentally infected reindeer.

1.3 STUDY AREA

The field work took place at Karrak Lake (67° 14' N, 100° 15' W) in the Queen Maud Gulf Bird Sanctuary, Nunavut, in the spring and summer of 2014-2015. Karrak Lake has the largest Ross's (*Chen rossi*) and lesser snow goose (*Chen caerulescens*) colony in the Sanctuary, consisting of nearly 1 million nesting geese in 2014 (R. T. Alisauskas, unpublished data). Since 1991, an ongoing study of the population ecology of arctic nesting waterfowl has occurred annually at this site (Alisauskas et al., 2012). In 2000, a study was initiated to investigate foraging behaviours and population dynamics of arctic foxes in relation to seasonal and annual variation in diet (Samelius, 2004).

1.3.1 Study species within Karrak Lake ecosystem

Arctic foxes are keystone inhabitants of the Karrak Lake ecosystem, where the fox population is unusually and relatively stable due to the seasonal abundance of geese and eggs. Arctic foxes are opportunistic predators and scavengers that generally rely on the abundance of small mammals (e.g., lemming and voles) for food, and therefore undergo dramatic population fluctuations following that of their prey. At Karrak Lake, birds and eggs can also constitute a major component of their diets, depending on the region and year (Bantle and Alisauskas, 1998; Samelius et al., 2007). When small mammals are scarce, the contribution of eggs from large goose colonies can increase within a range of 30-74% of their diet (Samelius et al., 2007). Arctic foxes will also scavenge or prey on other species like ptarmigan, arctic hare, muskoxen, and barren-ground caribou (Bantle and Alisauskas, 1998; Samelius et al., 2007). During winter, Arctic foxes have the ability to cover very large distances. Travel rates of 90km/day have been recorded previously (Tarroux et al., 2010). However, they tend to stay in the same area once settled (Samelius et al., 2007). Since Karrak Lake is approximately 60 km from the coast, it is possible that marine food items are present in their diet, especially when resources are scarce in the winter, or when foxes immigrate in the Karrak Lake ecosystem. However, Samelius et al. (2007) suggested little use of marine resources by studying stable isotope signatures in fox blood. The absence of parasites associated

with marine environments from Arctic fox feces is also consistent with a non-marine diet (Elmore et al., 2013).

At Karrak Lake, migratory geese have a considerable impact on Arctic fox survival and reproduction, leading to an unusually stable population of Arctic foxes over the years, with low den densities observed outside of the colony. With an estimate of more than 750, 000 Ross's and 450, 000 Lesser Snow Geese nesting in this region, this is one of the largest colonies in the Arctic, with nesting habitat spreading over 200 square kilometers (Alisauskas et al., 2012). Both species overwinter in the southern United States and migrate to breeding areas in the Canadian Arctic and Alaska from late May until August (Alisauskas et al., 2011; Elmore et al., 2014). Predators of geese in the Karrak Lake ecosystem include arctic foxes (*Alopex lagopus*), wolverines (*Gulo gulo*), wolves (*Canis lupus*), and barren-ground grizzly bears (*Ursus arctos horribilis*) (Bantle and Alisauskas, 1998; Wiebe et al., 2009; Elmore et al., 2014).

1.4 LITERATURE REVIEW

1.4.1 Life cycle of *Toxoplasma gondii*

Toxoplasma gondii was first described in 1908 in Tunisia within the tissues of *Ctenodactylus gundi*, a North African rodent (Nicolle and Manceaux, 1908). Its entire life cycle was completely understood only in the late 1960s, by discovering the key role of cats as a definitive host (Hutchison et al., 1969; Wallace, 1969; Dubey et al., 1970). In the same period of time, it was classified in the coccidian subclass, phylum Apicomplexa and the infectious parasitic stages (tachyzoites, bradyzoites, and oocysts) were also well characterized. Other genera of the phylum Apicomplexa involve important pathogens including *Plasmodium*, *Eimeria*, *Neospora*, *Babesia*, *Theileria* and *Cryptosporidium* (Kim and Weiss, 2004; Saeij et al., 2005).

The life cycle of *T. gondii* can be mostly summarized into two phases: 1) a sexual stage that occurs only within felids (domestic and wild cats), and 2) an asexual stage that can occur within virtually all

warm-blooded animals (Cleary et al., 2002). Because *T. gondii* can sexually reproduce only within the Felidae family, they are defined as the definitive host. All other hosts are defined as intermediate hosts (Dubey, 2004).

The three infective stages of *T. gondii* consist of a rapidly dividing invasive tachyzoite, a slowly dividing bradyzoite in tissue cysts, and an environmental stage, the sporozoite, protected inside an oocyst (Robert-Gangneux and Darde, 2012). Tachyzoites and bradyzoites result from asexual reproduction and can be produced in both intermediate and definitive hosts, while oocysts are the result of sexual reproduction and are produced in the intestinal epithelium of the definitive host (Dubey, 2009d). The sexual multiplication occurring in felids will result in the dissemination of large quantities of oocysts (egg-like structures) via feces in the environment (Dubey, 2009a). Over the course of a few weeks, a cat may shed millions of oocysts, which are highly infectious once sporulated and ingested by intermediate hosts and, to a lesser extent, other definitive hosts (Dubey, 1996a; Wendte et al., 2011). *Toxoplasma gondii* oocysts are highly resistant to environmental influences, including freezing (Jones and Dubey, 2010). In cold and dry climates, or aquatic environments, oocysts can survive and stay infective for many weeks (Dubey et al., 2011a). Studies have demonstrated that infective (sporulated) oocysts can survive up to 54 months in cold water (Dubey, 1998).

Following ingestion by a suitable vertebrate host, sporozoites (inside sporulated oocysts) convert to tachyzoites which multiply in the body, expanding the population of the parasite in the host and spreading via the bloodstream. This acute infection is normally controlled by the immune system, with the aid of interferon-g (IFN-g)-dependent mechanisms (Johnson et al., 1993; Saeij et al., 2005). The pressure from the host's immune system will cause tachyzoites to convert to bradyzoites, which are the semi-dormant and slowly dividing cellular stage of the parasite. They form tissue cysts that will predominantly persist in brain, eyes and striated muscles (including the heart) for the life of the host (Montoya and Liesenfeld, 2004). To reach immunologically privileged sites, where antigens are tolerated without eliciting an

inflammatory immune response, *T. gondii* has the capacity to cross non-permissive biological barriers (i.e., intestines, blood-brain barrier, and the placenta), thereby causing severe damage to those organs (Barragan and Sibley, 2003). However, specific tissue tropisms can vary among hosts (Dubey et al., 1998). Persistent bradyzoites can then initiate a new infection upon ingestion by a predator or scavenger. This parasite can thus be perpetuated asexually in this manner (Kim and Weiss, 2004). Tissue cysts can survive for several days after the death of an infected animal, even when the flesh starts decomposing (Work et al., 2000; Dubey, 2010b). They also survive storage at 4-6°C for up to 2 months but are usually destroyed by freezing at an internal temperature of -12°C, or when meat is cooked to an internal temperature of 66°C (Jacobs et al., 1960; Dubey et al., 1990b; Kotula et al., 1991; Dubey, 2010b).

1.4.2 Transmission

Three major routes of transmission of *T. gondii* in hosts have been described: congenital, faecal-oral, or by carnivorism (Dubey, 2009a). Congenitally acquired transmission occurs when an immunologically naïve mother becomes infected while pregnant. Symptoms in the foetus are usually more severe if infection occurs during the first trimester of pregnancy. No studies have found evidence for transmammary transmission in humans, but infective tachyzoites have been found in the milk of mice and cats, thus infecting the newborn via nursing (Pettersen, 1984; Powell et al., 2001). In humans, only a small percentage of infections are vertically transmitted (Tenter et al., 2000), with most people infected with *T. gondii* by ingesting oocysts shed by cats into the environment, or by consumption of infected meat products containing bradyzoites within tissue cysts (Kim and Weiss, 2008). Animals that graze, browse, and forage (e.g., cattle, sheep, goats, deer, birds, and poultry) are more likely infected by ingestion of oocysts from fecally contaminated environmental sources. Animals with carnivorous and omnivorous diets (e.g., dogs, cats, bear, boar, and other wildlife) can be infected either by ingestion of oocysts from the environment, or by consumption of infected meat. Overall, the tissue cyst stage of *T. gondii* functions in a prey-predator system that alternates between definitive (sexual reproduction) and intermediate (asexual replication) hosts (Robert-Gangneux and Darde, 2012). Once introduced into a food web, *T.*

gondii can be maintained when one intermediate host ingests tissue cysts from another, via carnivory or cannibalism.

Finally, on a much smaller scale, *T. gondii* can be transmitted through blood transfusions and organ transplants in humans, as well as through accidental injection in laboratories (Tenter et al., 2000; Hill and Dubey, 2002).

1.4.3 Toxoplasma as a zoonotic and veterinary pathogen

Toxoplasma gondii is ranked in the second highest category of biological agents that could cause serious epidemics in both human and animal populations (Gajadhar and Allen, 2004). In addition, according to the Food and Agriculture Organization report on risk management of foodborne parasites (FAO and WHO, 2012), it is also ranked as the 4th most important foodborne parasite globally after *Taenia solium*, *Echinococcus granulosus* and *Echinococcus multilocularis*. Even though it usually causes no or only mild symptoms, it can be life threatening for immunocompromised individuals, with encephalitis being the most important manifestation (Montoya and Liesenfeld, 2004). In women, congenital infection acquired during the first trimester can also cause devastating diseases for the foetus, ranging from mild ocular disease to severe hydrocephalus (Desmonts and Couvreur, 1974; Hill and Dubey, 2002). Serological diagnosis of congenital toxoplasmosis in newborns is usually made by looking for *T. gondii* specific IgM or IgA antibodies in the newborn (Naessens et al., 1999; Remington et al., 2004). Some studies have revealed a correlation between gestational age at the time of congenital infection and detection of IgM and IgA. If infected in the first half of pregnancy, often no antibodies are detected. This screening will more likely detect infected infants whose mothers were infected late in pregnancy (Foulon et al., 1999; Naessens et al., 1999). Moreover, manifestations of clinical symptoms in the infant are often absent at birth, but will more likely develop sequelae later on (Wilson et al., 1980). It has also been suggested that *T. gondii* may be a risk factor for developing schizophrenia and other psychotic disorders (Yolken and Torrey, 2008; Weiss and Dubey, 2009).

The clinical picture of toxoplasmosis in humans can strongly resemble those of non-human hosts (Dubey, 2010b), with most infections being subclinical or asymptomatic. However, some species seem to be more affected by the parasite than others, leading to severe clinical toxoplasmosis (Gustafsson et al., 1997; Brown et al., 2005; Pas and Dubey, 2008). Reasons for this remain unknown, but genetics, immune response, and a lack of co-evolution with cats has been suggested (Ketz-Riley et al., 2003; Brown et al., 2005; Maubon et al., 2008).

As a veterinary pathogen, vertical transmission remains of major importance, and *T. gondii* is a serious cause of fetal mortality in pig, sheep and goats (Dubey and Urban, 1990; Buxton, 1998; Duncanson et al., 2001; Dubey, 2009c; Hide et al., 2009; Innes et al., 2009). Vaccines are available to protect against *Toxoplasma* abortion in sheep and goats (Innes and Vermeulen, 2006). In order to help control the multiple routes of transmission of this parasite, a target strategy would include vaccination to prevent congenital toxoplasmosis, to reduce or eliminate tissue cysts, as well as vaccinating cats to prevent oocyst shedding, thus limiting the environmental contamination with the parasite, and reducing infection in intermediate hosts (Innes and Vermeulen, 2006; Innes et al., 2009).

1.4.4 Geographic Distribution

1.4.4.1 In Humans

Toxoplasma gondii has been found on all continents, including Antarctica. However, seroprevalence in humans and animals is usually lower in cold climates, in hot and arid areas, or at high elevation (Dubey, 2010b). In general, the degree of infection will vary depending on the population group as well as the geographic location (Montoya and Liesenfeld, 2004). Prevalences can vary widely between and within countries, from 4% in Korea to 92% in Brazil (Pappas et al., 2009; Dubey, 2010b). In northern Europe and the United States, studies have demonstrated a low seroprevalence compared to that in Central and South America (Tenter et al., 2000; Jones et al., 2001; Sroka et al., 2010). This high

prevalence is probably due to greater levels of oocysts in the environment, where cats are abundant and the climate favorable, as well as cultural, hygienic, and nutritional habits that can influence rates of exposure. People at lower socioeconomic levels often show a higher seroprevalence than at upper levels. Poor-hygiene conditions and water are thought to be an important source of human infection in disadvantaged areas, where the use of unfiltered surface water often prevails (Bahia-Oliveira et al., 2003; Dubey and Jones, 2008; Jones et al., 2008).

It is generally assumed that approximately a third of the world's human population is infected with *T. gondii* (Montoya and Liesenfeld, 2004). However, toxoplasmosis is often under-reported and under-diagnosed due to a lack of surveillance, as well as a lack of symptoms following infection most of the time (Barry et al., 2013). In Canada, one study estimated 4.0 million episodes of domestically acquired foodborne illness, with 2.4 million related to unspecified agents, including *T. gondii* (Thomas et al., 2013). According to their estimation, 9132 domestically acquired foodborne toxoplasmosis cases occur annually, with 293 cases presenting as ocular disease and 65 as symptomatic congenital infections. Although prone to negative bias, these estimates represent the most accurate and current values for an overall picture of toxoplasmosis in Canada. Moreover, estimates of seroprevalence in Indigenous communities in Canada have been reported as well (Tenter et al., 2000; Messier et al., 2009; Jenkins et al., 2013). In the United States, a national survey found a decrease in the age-adjusted *T. gondii* prevalence in U.S.-born people aged 12 to 49 years, from 14.1% in 1988 to 1994 to 9% in 1999 to 2004 (Jones et al., 2007). In fact, an increase in socioeconomic levels, in addition to an improvement of hygienic conditions, changes in farming systems, the consumption of frozen and cooked meat, and the feeding of cats with sterilized food, have led to a continuous decrease of the seroprevalence in most industrialized countries over the last decades (Robert-Gangneux and Darde, 2012). For example, a higher prevalence has been demonstrated in France, where undercooked meat is commonly eaten. However, better food hygiene as well as a change in food habits over the past few years have significantly decreased

the seroprevalence of *T. gondii* in this region (Tourdjman et al., 2015), hence the importance of public education.

1.4.4.2 In the Environment and Wildlife

Toxoplasma is widely prevalent around the world, especially in mild temperature climates, in part due to the survival of hardy oocysts in the environment (Dubey, 1998). The environmental reservoir of oocysts constitutes a considerable source of *T. gondii* infection, especially for herbivores that don't get infected via carnivorism (Dubey, 2010b). These hosts include domestic animals as well as game animals, both used for human consumption and therefore a risk of foodborne transmission. One study showed that sporulated *T. gondii* oocysts in soil could survive for 18 months in dry and moist conditions (Frenkel et al., 1975). However, it has been demonstrated that fewer oocysts survive in soil under dry conditions than under damp conditions (Lelu et al., 2012). In addition to foodborne transmission, *T. gondii* is a significant waterborne pathogen, with oocysts able to sporulate and survive in freshwater and marine environments for months (Dubey, 1998; Lindsay et al., 2003; Lindsay and Dubey, 2009). Nonsporulated oocysts can survive several weeks at 4°C and still be infectious when placed under appropriate conditions (Lindsay et al., 2002). Many reports exist of *T. gondii* infections in marine mammals including sea otters, dolphins, seals and whales (Dubey et al., 2003). Antibodies have even been detected in Antarctic pinnipeds, demonstrating how well established *Toxoplasma* is in marine ecosystems (Rengifo-Herrera et al., 2012). Surface water runoff has also been the source of infection and mortality in threatened sea otters in coastal environments (Miller et al., 2002; Conrad et al., 2005; Shapiro et al., 2010). Contamination of seawater and marine mammals may be more common than previously thought (Dubey et al., 2003; Dubey, 2004; Simon et al., 2013). For example, consumption of marine mammals and seafood has been found to be a risk factor for seropositivity to *T. gondii* in epidemiological studies in the Canadian North (Goyette et al., 2014). According to a Nunavik Inuit Health Survey done in 2004, frequent cleaning of water reservoirs and consuming untreated surface water is a risk factor for seropositivity to *T. gondii* in northern Canada. About one third of the population of Nunavik consumes untreated water (Messier et al., 2007). The

largest outbreak of acute toxoplasmosis in Canada has also been linked to a water reservoir in British Columbia, with the water source probably contaminated with oocysts from domestic and feral cats, and cougars (Bowie et al., 1997; Slifko et al., 2000). Many waterborne outbreaks caused by *T. gondii* have occurred in different parts of the world (de Moura et al., 2006; Palanisamy et al., 2006; Demar et al., 2007; Vaudaux et al., 2010; Baldursson and Karanis, 2011).

Historical temperature data demonstrated that the Mackenzie District and the Arctic Tundra climate region, which Karrak Lake is part of, is undergoing the highest warming trend in Canada, with a temperature increase of 2.2°C over the past 60 years (Hoberg et al., 2008). As a result, climate change might bring a range expansion of *T. gondii* via aquatic environments. Being highly resistant to external environmental conditions, *Toxoplasma* oocysts are easily disseminated in fresh or sea water. The oocyst's microscopic size and low specific gravity facilitate this mode of transmission. Their surfaces allow them to resist many chemicals commonly found in water systems. One study indicates that neither sodium hypochlorite nor ozone, commonly used in water public supplies, effectively inactivate *T. gondii* oocysts, even when used at high concentrations (Wainwright et al., 2007). Also, aquatic invertebrates might be responsible for outbreaks of toxoplasmosis in marine mammals (Gajadhar and Allen, 2004). The gills of filter-feeding oysters, clams, mussels and cockles can accumulate a significant amount of oocysts (Lindsay et al., 2001). One study demonstrated *T. gondii* oocyst infectivity in oysters for up to 85 days (Lindsay et al., 2004). Consumption of raw oysters, clams, or mussels has been determined to be a risk factor for human exposure to *T. gondii* (Jones et al., 2009; Goyette et al., 2014). As a result of climate change, the presence of invertebrate species in new areas that were previously too cool for their survival represents a risk for increased spread of pathogens, such as *Toxoplasma* (Gajadhar and Allen, 2004).

Over 350 species, including mammals and birds, have been described as hosts for *T. gondii* infection (Lindsay and Dubey, 2007; Robert-Gangneux and Darde, 2012). Most of the research has focused on humans and domestic animals (Wendte et al., 2011); however, recent studies in wildlife

demonstrate a wide host and geographic range and previously unrecognized genetic diversity of *T. gondii* in wildlife (Grigg and Sundar, 2009; Wendte et al., 2011). In addition to the originally identified Types I, II, and III strains (clonal lineages) of *T. gondii*, the identification of a high diversity of genotypes isolated from wildlife as well as a genetically distinct, dominant clone circulating in wildlife, ‘Type X’, raised questions concerning the extent and implications of the parasite genetic diversity circulating in wild animal populations and the degree to which sylvatic and domestic cycles are similar or distinct (Miller et al., 2008b; Dubey et al., 2011b; Wendte et al., 2011).

With the increase of urbanisation, humans and wildlife have more chances to interact. Wild animals are more likely to come into contact with environments contaminated with feces of both domestic and feral cats, increasing their chances of infection (Dubey et al., 2004; Dubey et al., 2011b). Moreover, scavengers, like bears and raccoons, could be good indicators of the prevalence of the parasite in the environment and its geographic distribution (Hill and Dubey, 2002). Likewise, sentinel aquatic organisms may serve as indicators of contamination of coastal environments (Simon et al., 2013). Arctic foxes studied in this research may also be used as indicators for potential human exposure and transmission of *T. gondii* in northern Canada.

1.4.4.3 In the Arctic

Antibodies to *T. gondii* in wildlife and people have been described throughout northern Canada, Alaska, eastern Greenland, as well as the Svalbard high Arctic Archipelago (Prestrud et al., 2008b; Prestrud et al., 2010; Jenkins et al., 2013; Goyette et al., 2014; Elmore et al., 2016b). Other transmission mechanisms are occurring in the tundra regions of the Arctic, where wild and domestic felids are rare. Lynx are usually not present and domestic felids are uncommon and rarely free-ranging above the tree line (Reichard et al., 2008). On the other hand, there can be transmission by oocysts occurring in boreal and sub-Arctic regions where free-ranging felids are present. Migratory intermediate hosts (such as

Arctic-nesting geese, barren-ground caribou, and marine mammals) can become infected through consumption of oocysts when they seasonally migrate into terrestrial or marine sub-Arctic environments contaminated with oocysts. Carnivores in arctic regions become infected through consumption of tissue cysts from migratory animals when they return up North (Prestrud et al., 2007).

Toxoplasma gondii has been described in multiple species throughout the circumpolar Arctic. Seropositivity in polar bears has been documented in Eastern Greenland (Rah et al., 2005; Oksanen et al., 2009), as well as Russia, Alaska and Canada. In the high Arctic of Svalbard, there is also a considerably high seroprevalence of infection in polar bears, ringed and bearded seals, and Arctic foxes (Prestrud et al., 2007; Prestrud et al., 2008b; Oksanen et al., 2009; Jensen et al., 2010). It has been suggested that *T. gondii* may have entered the terrestrial ecosystem via migratory birds (Sandstrom et al., 2013) since no wild felids are present and domestic cats are prohibited. In fact, Svalbard barnacle geese are exposed to the parasite, with a calculated seroprevalence of 7% (Prestrud et al., 2007). A similar scenario occurs in the Karrak Lake ecosystem, Nunavut, in the central Canadian Arctic. Ross's and Lesser Snow Geese as well as Arctic foxes have been commonly exposed to *T. gondii*, with seroprevalences of 39%, 36%, and 64% respectively (Elmore et al., 2014; Elmore et al., 2016b). Although pathology of *Toxoplasma* is rarely seen in the wild, acute disseminated toxoplasmosis in Arctic foxes has been reported in Svalbard (Sorensen et al., 2005). In wild ungulates of northern Canada, muskoxen and mainland caribou in the Northwest Territories and Nunavut have shown seropositivity for antibodies against *T. gondii*, with an overall seroprevalence of 6.4% and 37% respectively (Kutz et al., 2000; Kutz et al., 2001). It has also been reported in moose and caribou in Alaska and northern Quebec (Kocan et al., 1986; McDonald et al., 1990; Zarnke et al., 2000), and reindeer in Fennoscandia (Oksanen et al., 1997). One experimental infection in reindeer led to fatal enteritis (Oksanen et al., 1996), but no report of disease has been found in naturally infected reindeer or caribou in the wild. However, a *T. gondii* infection with encephalitis and placentitis was diagnosed in a full term stillborn reindeer fetus from the Houston Zoo, Texas (Dubey et al., 2002), becoming the only report of confirmed *T. gondii* natural infection in reindeer. Unfortunately,

little is known about effects on wildlife, but if *T. gondii* causes abortion and congenital disease in wild ungulates as it does in domestic livestock, failure of reproductive success can have an important impact on population of such species as caribou that are currently declining (Festa-Bianchet et al., 2011).

The significance of each route of infection (congenital, vertical, carnivory or waterborne) in the North is not well known. With little or no existence of felids in Arctic populations, oocyst transmission remains unlikely in terrestrial ecosystems, although water currents could be a possible dissemination route (Messier et al., 2009; Simon et al., 2013). Cultural habits of northern people likely affect the acquisition of *T. gondii* infection via ingestion of tissue cysts in undercooked meat (Hill and Dubey, 2002), knowing that Inuit populations have dietary preferences for raw, fermented, or dried meat (Messier et al., 2009). Seal, ptarmigan, and caribou are often consumed this way (McDonald et al., 1990). Other factors have been linked to infection, such as age, sex, schooling and community of residence. Skinning of animal for furs was also considered a risk factor for both seroconversion and seropositivity (McDonald et al., 1990). In Canada, studies have shown a 60% seroprevalence among the Nunavik general population, the only place where a screening program for detection of antibodies to *T. gondii* has been established in pregnant women (McDonald et al., 1990; Messier et al., 2009). The prevalence of individuals seropositive for *T. gondii* was higher in Nunavut (30%) compared with Inuvialuit and Nunatsiavut regions (8% and 11% respectively) (Goyette et al., 2014). Higher rates of infection in Inuit than in Cree communities in Northern Quebec have been reported, probably due to different dietary habits (Sampasa-Kanyinga et al., 2012; Goyette et al., 2014).

Despite the absence of definitive hosts, the occurrence of *T. gondii* in remote Arctic regions can be quite high. Cats play a crucial role in this parasite's ecology, but as we can see, it can exist in their absence. Undergoing rapid climate change and anthropogenic disturbance (ACIA, 2004), the North has been a region of great interest regarding emerging infectious diseases and interaction between people and wildlife (Hoberg et al., 2008). Further work is needed to fully understand the ecology of *T. gondii* in high

arctic environments. This thesis will address important questions regarding pathology of the parasite in caribou/reindeer, as well as innovative techniques to detect it in multiple arctic species. Moreover, looking at long term exposure in Arctic foxes and vertical transmission can help us understand how foxes are exposed, whether the parasite causes problems for the foxes, and how the parasite maintains itself in High Arctic ecosystems undergoing rapid environmental change. Finally, geese and caribou could also be sources of exposure for people who rely on hunting wildlife as culturally important and highly nutritious sources of food. Determining if the parasite is really present in tissue of these animals instead of prevalence via serological studies gives us important insights on food-borne transmission of *T. gondii* in northern communities.

CHAPTER 2: *Toxoplasma gondii* in Arctic foxes

Abstract

Transmission dynamics of *Toxoplasma gondii*, a parasite of importance for wildlife and human health, is enigmatic above the treeline in the Arctic, where the definitive host, free ranging felids and domestic cats, are absent or at very low density. We hypothesize that Arctic foxes are infected through consumption of migratory geese and caribou, and through vertical transmission to fox pups. We seek to determine the prevalence and timing of exposure through ongoing serological study of marked individuals in the field.

Fieldwork was performed in the Karrak Lake region within the Queen Maud Gulf Bird Sanctuary, in Nunavut, in the spring and early summer of both 2014 and 2015. For the collection of blood, adult foxes were chemically immobilized and pups were manually restrained. Animals were marked and observed over weeks to months in order to assess their behavior and diet.

Sera from 28 adult foxes (14 from 2014 and 14 from 2015) and from 30 fox pups (18 from 2014 and 12 from 2015) were tested via two serological methods, Indirect Fluorescent Antibody Test (IFAT) and Modified Agglutination Test (MAT) for antibodies to *T. gondii*. There was 100% agreement between the two tests when read by a blinded viewer. In 2014, 8 of 14 adult Arctic foxes, and 1 of 18 pups, were seropositive. For summer 2015, 7 of 14 adults, and 6 of 12 pups, were seropositive.

By looking at long term exposure of Arctic foxes, we demonstrated that mature foxes are more likely to be exposed and seroconvert than young foxes. Mid-aged foxes (2-4 years) had the highest rate of seropositivity. In one older fox, antibodies faded away, implying that *T. gondii* antibodies might fluctuate in time. Moreover, this study showed for the first time the possibility of congenital infection in naturally infected Arctic foxes to their pups, meaning that vertical transmission might play a role in exposure to *T. gondii* in the Karrak Lake ecosystem. While transfer of maternal antibodies through milk or in utero remains a possible explanation for the presence of antibodies in pups, there were 15 similar age,

seronegative pups from 3 different seropositive mothers. Finally, we detected antibodies in pups from seropositive mothers that had antibodies at least a year before giving birth, suggesting that subsequent litters could possibly be infected congenitally. This is contrary to the paradigm that only females infected for the first time in pregnancy can infect their progeny.

2.1 INTRODUCTION

Arctic foxes (*Vulpes lagopus*) have a circumpolar distribution throughout Arctic tundra habitats. They are commonly found in Alaska, Canada, Svalbard, Greenland, Iceland, and Siberia (Norén et al., 2011). Their population ecological characteristics (i.e., distribution, density, dispersion, growth rate, and age structure) as well as their prey (i.e., rodents, birds, marine resources, eggs, reindeer, and muskoxen) vary depending on the region they are found in. Their current population trend is described as stable, with a world population in the order of several hundred thousand animals (Angerbjörn and Tannerfeldt, 2014). Although the Karrak Lake fox population appears to be stable, elsewhere in the world Arctic foxes are of conservation concern due to the effects of environmental change and competition with other carnivores, such as red foxes moving north (Hersteinsson and MacDonald, 1992; Pamperin et al., 2006). The arctic fox is classified as critically endangered in Finland, Norway and Sweden, and within the European Union (Angerbjörn et al., 2012).

Mortality of Arctic foxes from *T. gondii* has occurred in other parts of the world, such as reported in the Svalbard, Norway population (Sorensen et al., 2005). Acute disseminated *T. gondii* infection was identified in three wild arctic foxes found dead in the same geographic area of Svalbard. One of them had concurrent infection with *Y. pseudotuberculosis* and *Salmonella enteritidis*, which may have contributed to the severity of the disease. No predisposing factors were found in the two other foxes. In this population, a seroprevalence as high as 43% has been found in 594 foxes tested (Prestrud et al., 2007). Similar to the Karrak Lake ecosystem, migratory birds are thought to be an important source of *T. gondii* in this region, with a seroprevalence of 7% in barnacle geese. Prestrud et al., (2007) also found a higher prevalence in < 1-year-old foxes, with the youngest foxes assayed being 6-9 month old, indicating that vertical transmission could be an important method of transmission in Arctic foxes. In the Karrak Lake fox population, mature foxes had higher antibody prevalence estimates than yearlings; however, some

young foxes (≤ 1 year) were antibody positive, even though they have lower probability of foodborne exposure (Elmore et al., 2016b). This may be due to vertical transmission.

Vertical transmission of *T. gondii* is well established in domestic animals and captive wildlife. High levels of congenital infection have been demonstrated in wild populations of mice, as well as in sheep, and may be more widespread than previously thought in a natural population (Tenter et al., 2000; Duncanson et al., 2001; Marshall et al., 2004; Hide et al., 2009). Experimentally infected blue foxes (*Alopex lagopus*) have shown a high mortality rate in newborn pups born with acute systemic toxoplasmosis (Bjerkas, 1990). Reports of infected vixens and death of neonates has been demonstrated in multiple farm foxes as well (Smielewska-Los et al., 2000). However, no study has looked into vertical transmission of the parasite in wild Arctic foxes, by comparing seroprevalence in both mothers and pups newly emerged from their dens.

Since 2000, studies on Arctic foxes have been conducted at Karrak Lake, Nunavut, focusing on foraging behavior and population dynamics in relation to variation in foods (Samelius, 2004; Samelius et al., 2007). Starting in 2011, blood from live-trapped Arctic foxes has been collected and analyzed for *Toxoplasma* every year (Elmore et al., 2016b). This on-going project is an opportunity to monitor disease transmission dynamics in an unusually stable, natural population of Arctic fox. *Toxoplasma* is a parasite that has developmental stages that live in tissues and to diagnose these parasites would require invasive or post-mortem techniques for detection. The collection of blood and subsequent analysis by common serological techniques can determine if an animal has been exposed. This information is valuable for research that aims to identify potential risks to human and animal health in a vulnerable Arctic ecosystem.

2.2 OBJECTIVES

- To investigate vertical transmission of *Toxoplasma gondii* in Arctic foxes (i.e., female foxes to the pups).
- To explore interannual dynamics of *Toxoplasma gondii* in Arctic foxes in a long term serostudy.
- To compare serological techniques (MAT vs IFAT) in Arctic fox blood samples.

2.3 METHODS

2.3.1 Study area

In May 2014 and 2015, adult Arctic foxes were trapped in the Karrak Lake region (67°14' N, 100°15' W), within the Queen Maud Gulf Bird Sanctuary, in Nunavut (see Figure 1). Over one million Ross's Geese (*Chen rossii*) and Lesser Snow Geese (*Chen caerulescens*) nest annually in this area, making this colony one of the largest on Earth (Kerbes et al., 2006; Alisauskas et al., 2012). Arctic foxes of this region rely heavily on small mammals, including collared lemmings (*Dicrostonyx groenlandicus*), brown lemmings (*Lemmus sibiricus*) and red-backed voles (*Clethrionomys rutilus*). However, other food sources like birds and eggs are an important part of their diet as well, especially when small mammals are scarce. Goose eggs could contribute up to 60% of all foods taken by foxes during goose-nesting at Karrak Lake (Samelius et al., 2007). Nevertheless, lemming abundance remains the main factor driving fluctuation of Arctic fox population and reproduction, even with seasonally abundant foods available, such as geese (Samelius et al., 2011).

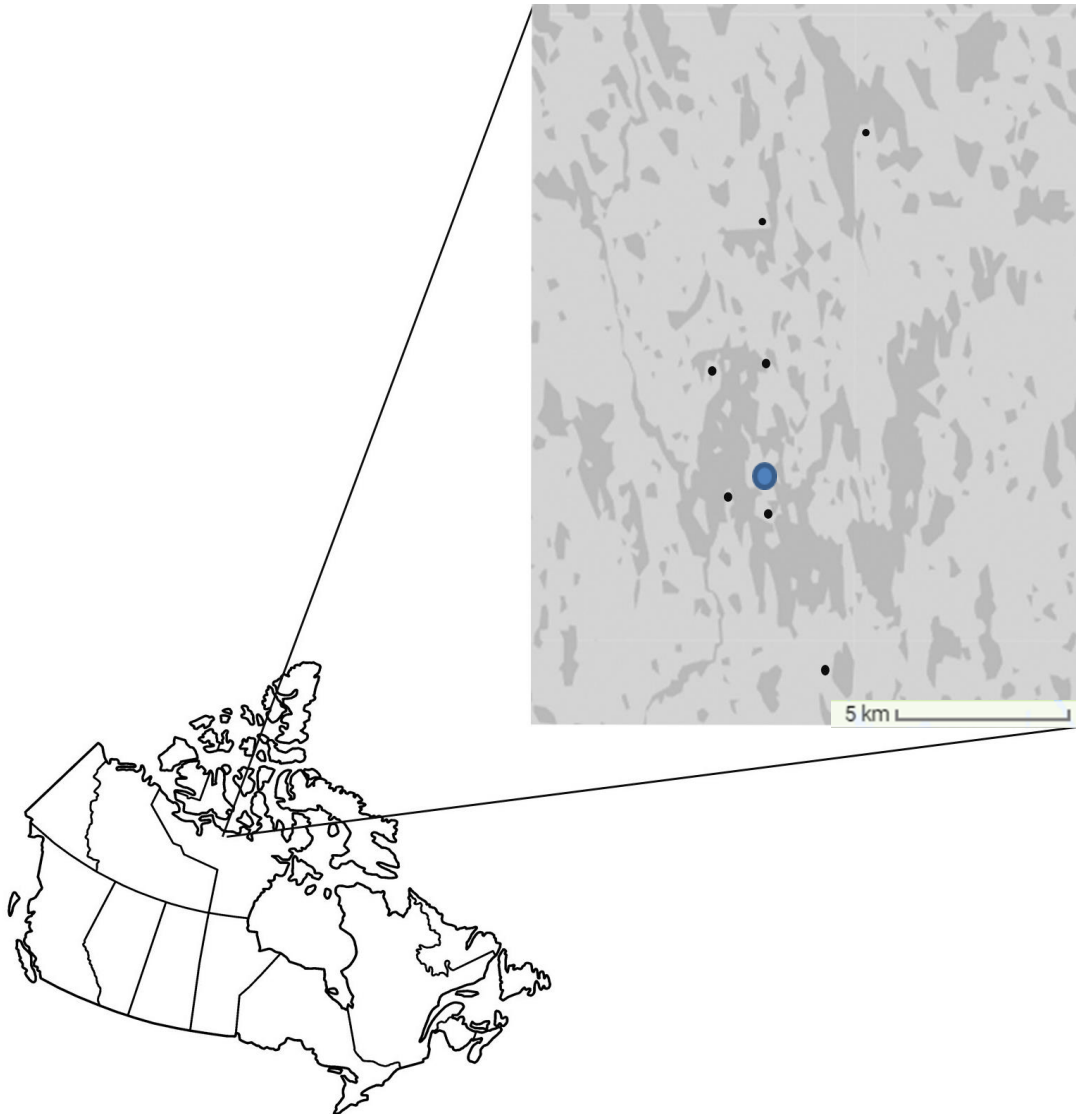


Figure 1. Location of the Karrak Lake goose colony in Nunavut, Canada. Square represents trapping area within the colony and dots are Arctic fox dens with trapping success in 2014-2015. Bigger dot represents the field camp.

2.3.2 Fox trapping and blood sampling

Adult arctic foxes were captured in spring of 2014 and 2015 ($n = 28$) by using box-traps, a collapsible one-door live animal cage trap (Havahart model 1089, Woodstream Corporation, Lititz, Pa.) baited with sardines. Traps were left open and baited a few days prior to capture to accustom foxes and improve capture success. The traps were placed at locations with signs of fox activity (e.g., den sites, elevated knolls and large rocks) in a 5×14 km area of the original and central part of the goose colony starting in May. We checked traps continually from inside a tent (serving as a blind) placed a few meters from the

trap, and removed captured foxes by flushing them into a net placed over the trap's entrance. Foxes were restrained by hand with thick gloves to avoid injuries. In 2015, we improved the capture technique by using a wood press to immobilize the fox against the back of the trap, and injecting the drug through the wire netting. We injected 15 mg of Telazol (corresponding to 0.15 ml of the solution reconstituted to 100 mg/ml) intramuscularly into the upper part of the back leg (mean induction time of 2.4 min). No ill-effects of the drug have been seen previously and immobilisation with Telazol is characterised by safe handling and predictable recovery (Samelius et al., 2003). Foxes were individually marked with permanent plastic ear-tags (Dalton ID Systems Ltd., 1×3.5 cm in size), weighed, sexed, and the right hind-foot was measured. Blood samples were collected in 5ml serum separator tubes from the cephalic vein on the lower front leg (ca. 2ml) by using 3ml syringes and 23 gauge needles. This procedure was done quickly, in silence, to minimize stress. Blood was centrifuged at 8000 rpm for 20 minutes and sera stored at -20°C at the field camp.

Juvenile arctic foxes (pups) were captured starting in June in the summers of 2014 and 2015 (n=30). Capturing and marking of arctic fox pups is a common technique used worldwide with no reports of ill effects to foxes (Meijer et al., 2011). Pups were trapped, sexed, and tagged as per adult foxes, except chemical immobilization was not necessary and blood (ca 2ml) was collected from the jugular (vs cephalic) vein in 5ml serum separator tubes using 3ml syringes and 23 gauge needles. Blood was centrifuged at 8000 rpm for 20 minutes and sera stored at -20°C at the field camp.

These procedures were approved by the University of Saskatchewan Animal Care Committee for the blood collection and live-trapping (UCACS protocol number 20100159/19990029) and follow the Guidelines of the Canadian Council on Animal Care. We also held wildlife research permits from the

Nunavut Department of Environment 2014-029/2015-019 and Canadian Wildlife Service NUN-MBS-14-02, NUN-SCI-14-02.

2.3.3 Serological methods

Each serum sample was tested using two different serological analyses.

2.3.3.1 Modified Agglutination Test (MAT)

A new commercially available kit (New Life Diagnostic LLC, Carlsbad, CA, United States) (Al-Adhami et al., 2016) was used according to the manufacturer instructions. Three dilutions were performed on fox serum (1:25-1:50-1:100). On a U bottom 96 well assay plate, 25µl of antigen mixture was mixed with 25µl of serum dilutions and incubated overnight. A blue button at the bottom of the well indicated a negative result.

2.3.3.2 Indirect Fluorescent Antibody Test (IFAT)

Following manufacturer's instructions (VMRD, Pullman, WA, USA), the diluted fox serum (1:50) was applied on slides containing the antigen. After incubation at 37°C for 30 minutes, the slides were rinsed with a rinse buffer (pH=9). Anti-canine IgG antibodies conjugated to fluorescein isothiocyanate (FITC; rabbit origin) was applied on slides. After incubation and rinsing, slides were viewed under Olympus DP70 fluorescence microscope, at 40X objective.

2.3.4 Aging method

Foxes were categorized under three different age ranges: young (≤ 1 year), mid-aged (2-4 years), and older (≥ 5 years). Age was estimated by studying the teeth condition, whether the teeth were not worn, slightly worn, or worn (G. Samelius, unpublished data), as well as examining the recapture data (Elmore et al., 2016b).

2.3.5 Data analysis

Both tests were read by the same person, and performed at different times. Numbers were given to the samples in order to keep the reader blinded in between assays. We estimated the apparent seroprevalence by dividing the number of positive foxes that were clearly *T. gondii* seropositive by the total number of samples tested. Statistical analyses were performed using IBM SPSS Statistics 20. The agreements between the MAT and IFAT were determined by the kappa coefficient (κ). For IFAT, a complete staining around the tachyzoites was considered positive for *T. gondii* antibodies. Tachyzoites with little, discontinuous, or no staining were recorded negative. For MAT, a clear bottom with agglutination covering 50-100% of the well was considered positive.

2.4 RESULTS

A total of 28 adult foxes were captured in 2014 and 2015, including six recaptures in 2015 (22 individual foxes in total). For fox pups, 12 were captured in 2014, and 18 in 2015. In 2014, the apparent seroprevalence in adult Arctic foxes ($n = 14$) was 57.1% (95% CI = 0.27-0.87) for both MAT and IFAT, and 5.6% (95% CI = 0.00-0.17) for juvenile Arctic foxes ($n = 18$) for both tests as well. In 2015, the apparent seroprevalence in adult Arctic foxes ($n = 14$) was 50.0% (95% CI = 0.20-0.80) for both MAT and IFAT, and 50.0% (95% CI = 0.17-0.83) for juveniles ($n = 18$) for both tests. The results are illustrated in Table 1.1 and 1.2. Kappa coefficient for MAT and IFAT was equal to 1 since no difference was found between both tests.

Table 1.1

Serological prevalence of *T. gondii* antibodies in adult Arctic foxes by MAT and IFAT for 2014-2015

Assay		Antigen	Sample size	Positive		Negative	
				No.	%	No.	%
MAT/IFAT	2014	Whole tachyzoite antigen	14	8	57.1	6	42.9
	2015		14	7	50.0	7	50.0

Table 1.2

Serological prevalence of *T. gondii* antibodies in juvenile Arctic foxes by MAT and IFAT for 2014-2015

Assay		Antigen	Sample size	Positive		Negative	
				No.	%	No.	%
MAT/IFAT	2014	Whole tachyzoite antigen	18	1	5.6	17	94.4
	2015		12	6	50.0	6	50.0

Arctic foxes have been trapped since 2011 at this location, with a total of 13 recaptures throughout the years, and blood tested for antibodies to *T. gondii* using IFAT and a commercially available MAT kit (ToxoScreen-DA, Biomerieux, Marcy l'Etoile, France). Table 2 illustrates the long term exposure of all the adult foxes trapped and recaptured in the past 5 years, including adult foxes trapped in 2014 and 2015 in the current study.

Table 2

Long term trends in seroprevalence for *T. gondii* antibodies in individual, marked adult Arctic foxes at Karrak Lake

Fox identifiers IFAT/MAT	Age range (estimated in 2015)	(Elmore, unpubl data.)			2014	2015
		2011	2012	2013		
OYPO ♂	Older	-	-	-	-	-
PYOY ♀	Mid-aged	n/a	n/a	+	+	+
OYYY ♀	Older	n/a	n/a	-	+	+
OOGG ♂	Older	n/a	+	+	n/a	n/a
PPYO ♂	Older	-	+	n/a	+	+
PYYO ♀	Mid-aged	n/a	-	n/a	+	+
YPPY ♀	Mid-aged	n/a	+	+	+	+
OOPP ♀	Older	+	n/a	n/a	-	n/a
PPYY ♂	Mid-aged	n/a	n/a	-	-	n/a
YYOO ♂	Mid-aged	n/a	n/a	+	+	dead
POPP ♀	Mid-aged	n/a	-	+	+	n/a
*OGPO ♂	Mid-aged	n/a	n/a	n/a	-	n/a
*OGPP ♂	Mid-aged	n/a	n/a	n/a	-	n/a
*GOPO ♂	Young	n/a	n/a	n/a	+	n/a
*OGPY ♂	Young	n/a	n/a	n/a	-	n/a
YYGO ♀	Mid-aged	n/a	n/a	-	n/a	-
PYYY ♂	Older	n/a	n/a	+	n/a	+
*GGPG ♂	Mid-aged	n/a	n/a	n/a	n/a	-
*GOPY ♀	Mid-aged	n/a	n/a	n/a	n/a	+
*GPGO ♀	Young	n/a	n/a	n/a	n/a	-
*PPPP ♀	Older	n/a	n/a	n/a	n/a	-
*YYYY ♂	Young	n/a	n/a	n/a	n/a	-
*OOOO ♂	Young	n/a	n/a	n/a	n/a	-

* Captured only once

For 2014 and 2015, 3/6 older foxes, 6/11 mid-aged and 1/5 young were seropositive. Four foxes seroconverted over the course of the study. One was older, two were mid-aged, and one was young at the time of seroconversion. In one initially mid-aged seropositive fox, antibodies faded away. Two foxes stayed positive for at least 3-4 years. Finally, Table 3 demonstrates the seroprevalence in the adults and their respective litter for both field seasons using MAT and IFAT.

Table 3
Seroprevalence of *T. gondii* in parents vs litter using IFAT and MAT
for summer 2014 and 2015

2014	IFAT/MAT	
	Adults	Pups
PYOY ♀ OYPO ♂	+ -	1 positive on 7 trapped (litter of 7)
POPP ♀ PPYY ♂	+ -	0 positive on 6 trapped (litter of 7)
OYYY ♀ PPYO ♂	+ +	0 positive on 5 trapped (litter of 9)
2015	IFAT/MAT	
	Adults	Pups
PYOY ♀ OYPO ♂	+ -	0 positive on 4 trapped (litter of 7)
PPPP ♀ Unknown ♂	- ?	0 positive on 2 trapped (litter of 5)
PYYO ♀ PYYY ♂	+ +	6 positive on 6 trapped (litter of 10)

2.5 DISCUSSION

2.5.1 Vertical transmission of *T. gondii* in a free-ranging population

We demonstrate, for the first time, the possibility of natural vertical infection of *T. gondii* in neonate Arctic foxes, from seropositive female foxes to their pups. All seropositive pups caught were still nursing, which makes it less likely that were infected by foodborne transmission. Vertical transmission might thus represent another way (besides foodborne) for the parasite to transmit among Arctic carnivores. Both litters with positive pups were from mothers who had been seropositive the year previously. This suggests that subsequent litters can possibly be infected congenitally, not just litters of females infected for the first time in pregnancy, which may have greater significance for lifetime reproductive success. These findings have also been reported in deer mice with congenital infection in multiple litters (Rejmanek et al., 2010). Previously it was assumed that pups only become infected when the female is infected for the first time during pregnancy and lactation, as is usually the case for other mammals, including people (Roberts and Alexander, 1992; Dunn et al., 1999; Tenter et al., 2000; Thiebaut et al., 2007; Moncada and Montoya, 2012). Trans-placental infection rarely occurs when *T. gondii* is acquired prior to gestation (Remington et al., 2004; Rahman et al., 2015). For two seropositive mothers in 2014, none of the pups were seropositive, suggesting that these females were exposed prior to becoming pregnant. These females became seropositive for *T. gondii* in 2013 and 2014. None of the pups were seropositive from the one seronegative female for which the serostatus of the pups was determined.

One of 7 pups was seropositive from a seropositive female in 2014. This female was not acutely infected in 2014, as she was seropositive in 2013. Interestingly, she went on to have seronegative pups in 2015, suggesting that risk of congenital infection decreases with subsequent litters. Having only a few members of a litter seroconvert is a common finding in dogs infected with *Neospora caninum*, closely related to *T. gondii*. Multiple litters can also be infected with *Neospora* (Dubey et al., 1990a). Some studies suggest that after primary infection, some degree of host immunity develops over time, impeding

infection to the foetus in subsequent pregnancies (Dubey et al., 2007; Donahoe et al., 2015). However, multiple litter infections with *Toxoplasma* are not the norm. Although repeat transmission has been demonstrated in sheep (Morley et al., 2008; Hide et al., 2009; Dubey, 2010b), it remains infrequent most of the time.

It is possible that seropositive pups represent false positives due to maternal IgG antibodies passively transferred in utero to the foetus, or through the colostrum/milk (Omata et al., 1994; Remington et al., 2004). Maternal antibodies represent an important part of circulating IgG in juvenile animals. The detection of IgG usually stops abruptly as soon as weaning takes place (Van de Perre, 2003). From the one seropositive pup in 2014, we collected blood on June 18. Pups emerge from the den after 3–4 weeks, and are weaned at 6–7 weeks (Audet et al., 2002). We observed this whole litter coming out of the den on mid-June. Therefore, we estimated the age of this pup at around 5 weeks. No previous study tested wild fox pups as soon as they emerge from the den. The age estimate of the other pups trapped and tested from this den ranged from 5-9 weeks, with the last pup trapped on July 15. No other pups were seropositive, even those trapped within less than a week from the seropositive, making it unlikely that maternal antibodies were responsible for the seropositive (Omata et al., 1994; Casal et al., 1996). For the seropositive pups caught in 2015, a whole litter demonstrated IgG antibodies against *T. gondii*. The first one was caught on June 28, and the last one on July 4, estimating their age in between 6-8 weeks. Transfer of maternal antibodies could be a possibility, but we would expect lower detection of IgG at 8 weeks of age (Omata et al., 1994).

It is not clear if vertical transmission in this population would be transplacental or transmammary, as we were not able to sample pups at birth. In experimentally infected cats, it has been demonstrated that tachyzoites of *T. gondii* can be transmitted via the milk of the mother to the youngsters (Powell et al., 2001). Tachyzoites also have been found in the milk of sheep, goats, cows and mice (Pettersen, 1984; Skinner et al., 1990; Johnson, 1997; Tenter et al., 2000; da Silva et al., 2015). No studies report

congenital toxoplasmosis in dogs. However, transmission has been demonstrated in experimental infections of pregnant bitches (Bresciani et al., 1999; Bresciani et al., 2009). Therefore, both types of vertical transmission could occur. Transmission of tachyzoites to the female Arctic fox by the male through semen remains also a possibility of infection for female foxes and pups. However, we were unable to explore this hypothesis as the sire was unknown for the only seronegative female fox for which we tested kits. One study demonstrated vertical transmission in dogs via semen by infecting male dogs and artificially inseminating female dogs with *T. gondii* positive seminal samples. Several *T. gondii* cysts were detected in the brains of four offspring (Arantes et al., 2009).

2.5.2 Dynamics of transmission of *T. gondii* in a free-ranging population

One previous study estimated seroprevalence for *T. gondii* antibodies in adult Arctic foxes in the Karrak Lake region at about half of trapped animals (Elmore et al., 2016b). This estimate remains similar for the data collected in this study, with an overall prevalence of 54% (95% CI = 0.37-0.73) in adults for both years. Live trapping, tagging, and monitoring for antibodies to *T. gondii* in adult Arctic foxes at this location for the past 5 years demonstrates that overall seroprevalence remains mostly stable throughout the years (Table 2). A stable seroprevalence in the Karrak Lake fox population would indicate a constant infection pressure from their environment (Opsteegh et al., 2011), meaning that they are routinely exposed to *T. gondii*, whether it is by trophic or vertical transmission. However, changes in serostatus of individual, recaptured foxes can provide insight into timing of exposure and antibody persistence in naturally infected animals. Four foxes seroconverted over the course of the study, including three mature foxes, and one young fox at the time of seroconversion. For 2014 and 2015, 3/6 older foxes, 6/11 mid-aged and 1/5 young were seropositive. Mid-aged and older foxes are thus more likely to be exposed and seroconvert than young foxes. Several animals stayed seropositive for at least 3-4 years, suggesting antibody persistence and/or re-exposure. Finally, in one initially mid-aged fox who was seropositive in 2011, antibodies were not present when this animal was recaptured and tested in 2014. Since we don't

have data between 2011 and 2014, we cannot say exactly when this fox became seronegative; however, it was classified as older in 2014. Antibodies against *Toxoplasma* are usually thought to persist for a lifetime in its host, probably due to the constant antigenic stimulation from persistent tissue cysts (Remington and Krahenbuhl, 1982). Interestingly, one older fox remained seronegative for the past five years, which could mean that this fox had been infected earlier in life and antibodies had faded, or this fox was never exposed, possibly due to dietary preference. Although our sample size is not sufficient to understand fully the dynamics of antibody persistence, ongoing study of prevalence of *T. gondii* antibodies in this fox population will give a better idea of when foxes get exposed, how antibody production changes, and if any problems (i.e., low reproductive success) are correlated with chronic *T. gondii* infection in this Arctic fox population.

Our sample size is too small to determine if reproductive success is influenced by *T. gondii*. However, of the 14 adults trapped in 2014, 3/7 females gave birth, and for summer 2015, 4/9 females gave birth. This is a reproductive success rate of 43% in 2014 with an average of 8 pups per litter, and 44% in 2015 with an average of 7 pups per litter. The Arctic fox has one of the largest litter sizes in the Carnivora order, but it can be extremely variable depending on food availability (Tannerfeldt and Angerbjörn, 1998; Fuglei et al., 2003; Meijer et al., 2011). Litter size can range from 3 to 25 pups. Litter sizes of 6–12 young are common for inland Arctic foxes, whereas coastal fox litters more commonly average 6 pups (Audet et al., 2002). It is possible that our reproductive success estimate is low, as some trapped females could have given birth outside the research area. Moreover, it is not unusual in low lemming years for foxes to have lower reproductive success. We believe 2014 was the beginning of an increase in lemming abundance.

2.5.3 Comparison of serological tests for *T. gondii* in a wild population

Serological studies, especially in wildlife for which these tests are rarely optimized, are inherently limited; even a positive result only tells us that the host has been infected at one point in its life, and is not necessarily actively infected (Dubey, 2010b). Numerous serological tests for *T. gondii* exist to detect IgG and IgM, the two main antibodies found in the blood after infection. IgM is often considered as a sign of an acute infection as IgM are the first antibodies to be produced by the body, generally within 1 week following infection. IgG will usually be detectable at about 2 weeks after the infection and continue to increase at least 6 weeks post infection (Lin and Bowman, 1991; Lin et al., 1992).

To diagnose an acute *T. gondii* infection, ideally one should demonstrate a rise in IgG antibody titres in serial serum samples – i.e., IgG avidity assays (Lappalainen and Hedman, 2004). Unfortunately, having more than one blood sample from the same individual a few weeks apart would be very hard to achieve when trapping wild animals. IgG avidity test is often performed in addition to confirm an IgM positive test when looking for congenital toxoplasmosis. We did not test for IgM antibodies in this study. Heavier IgM antibodies do not cross the placenta, whereas the lighter IgG antibodies do (Dubey, 2010b). IgM are known to give a lot of false positives and can be unreliable with a low specificity (Robert-Gangneux and Darde, 2012), thus the necessity of a second technique to confirm the diagnosis. Although considered a marker of acute infection, IgM can also persist for years after infection, and not all neonates produce detectable levels of IgM (Naessens et al., 1999; Lappalainen and Hedman, 2004).

Ideally, diagnosis of *T. gondii* infection should not rely on a single serological test (Li et al., 2016). Other methods might be necessary to confirm infection, such as polymerase chain reaction (PCR) on tissues, histological demonstration of the parasite and/or antigens, or isolation of the organism itself by using mouse bioassay (Dubey, 2010b); unfortunately, none of these tests can be used antemortem in free ranging wildlife. In live wildlife hosts, serological methods remain the easiest way to determine exposure

to many pathogens. However, known positive and negative controls, as well as a gold standard diagnostic assay are often not available for wildlife species, bringing uncertainty when interpreting results (Peel et al., 2013). For these reasons, we chose to use more than one serological assay to confirm exposure status.

This study compared IFAT and MAT for their ability to detect *T. gondii* antibodies in naturally infected Arctic foxes. These methods were used due to the ease of their preparation and application as well as due to their high detection probability when compared with other test methods (Elmore et al., 2016b). For this research, both tests correlated well, with 100% agreement, making us confident in our ability to accurately determine exposure status. One study compared IFAT and MAT, and reported higher sensitivity (97.8%) in cat serum by MAT, when compared to IFAT, than dog serum (73.4%) (Macri et al., 2009). Both assays need visual inspection in order to determine serostatus of the samples, which can bring potential biases when reporting the results. In another study, data for serum samples collected from wildlife including foxes, and tested for *T. gondii* by this new MAT indicated high sensitivity of the test when compared to the older commercial one (Al-Adhami et al., 2016). It is possible that cross-reaction with other coccidians might be occurring with these serological methods (Dubey and Lindsay, 1996). *Neospora caninum* is a coccidian closely related to *T. gondii*, and dual infections are commonly found (Dubey, 2010b). However, specificity of the MAT used in our study is estimated to be *T. gondii*-specific at 90% according to the manufacturer's instructions (New Life Diagnostic LLC, Carlsbad, CA, United States).

A third method could have been used in order to increase our certainty about exposure status. Enzyme-linked immunosorbent assay (ELISA) is another serological test that can be used to detect *T. gondii* antibodies. However, since ELISA generally requires a taxon-specific secondary conjugate antibody, and we lacked fox reference sera (known negative and positive samples to set cut-off values), this test was not performed (Elmore et al., 2016b). An indirect ELISA using a protein A/G conjugate generalized to all mammals has been developed to overcome the taxon-specific antibody requirement (Al-Adhami and Gajadhar, 2014). However, we still lacked the controls needed to set cutoff values.

2.5.4 Summary

This work is part of a long term study that will give us a better understanding of how foxes are exposed to *T. gondii*, the dynamics of antibody persistence, whether the parasite causes problems for the foxes, and how the parasite maintains itself in High Arctic ecosystems undergoing rapid environmental change. Mature foxes (≥ 2 yr) demonstrated the highest seroprevalence of antibodies to *T. gondii*. Seroconversion was also more likely to occur in mid-aged foxes. In one mature fox, antibodies faded away, meaning that *T. gondii* antibodies might fall in the absence of re-exposure, which is contrary to the paradigm that antibodies persist for life. Furthermore, this work demonstrated for the first time the possibility of congenital infection in naturally infected Arctic foxes to their pups, and that vertical transmission might not be limited to litters of mothers exposed to *T. gondii* for the first time during that pregnancy. Further work is needed to determine the longevity of maternal antibodies, if the whole litter is usually infected, if infection commonly occurs in multiple litters, and what effects infection has on pup survival inside the mother and in early life.

CHAPTER 3: *Toxoplasma gondii* in Arctic-nesting geese

Transition statement

In the previous chapter, I demonstrate that Arctic foxes in the Karrak Lake ecosystem are exposed to *T. gondii* and transmission may occur vertically as well as through food-borne routes. Since migratory geese are an important part of the diet of these foxes, and previous work has shown that geese at Karrak Lake are exposed to *T. gondii*, I sought to detect *T. gondii* in tissues of geese harvested at Karrak Lake. Since there are no gold standard diagnostic tests for this parasite in wildlife, I also compared molecular and serological methods for detection, and performance of a serological test in the field. The latter is important as a potential “carcass-side test” to estimate *T. gondii* exposure in Arctic-nesting geese which are hunted and consumed by people in the Arctic and elsewhere along their migratory route. Genetic characterization of isolates of *T. gondii* from geese would also give us information on how the parasite is introduced and maintained in this ecosystem.

Abstract

In North America, there is a lack of surveillance for *T. gondii* in wildlife and people, despite the fact that Inuit in the Eastern Canadian Arctic have some of the highest levels of exposure observed in North America. The source of this parasite in Arctic tundra regions is somewhat mysterious, since the usual definitive hosts for this parasite are domestic cats, which are rare in the Far North. Previous work shows that the parasite might be introduced by migratory geese, millions of which nest in the Canadian Arctic each summer and could be exposed to the parasite on their wintering grounds and during migration. Geese and other migratory wildlife (like marine mammals and caribou) could also be sources of exposure for people who rely on hunting wildlife as culturally important and highly nutritious sources of food.

Our objective was to determine presence of *T. gondii* in wild Arctic-nesting geese in Karrak Lake ecosystem using serological and molecular methods. We compared results from shot geese using a serological method, a new commercially available modified agglutination test (MAT), on 1) fresh thoracic fluid on a subset of geese in the field (detection in 1/5 Ross's and 2/16 Lesser Snow Geese), 2) frozen thoracic fluid thawed in the laboratory (14.8% of 27 and 9.5% of 21, respectively), 3) blood thawed from frozen hearts in the laboratory (3.1% of 32 and 3.4% of 29, respectively), and 4) filter papers dipped in thoracic fluid in the field, dried, then frozen (0% of 20 and 0% of 21, respectively). Of the three geese that tested positive in the field on fresh thoracic fluid, only one was positive in the laboratory; conversely, one goose that tested negative in the field was positive when frozen thoracic fluid was tested in the laboratory. Our results suggest that performing the MAT in the field is feasible, and provides the best results, and that common methods of collecting and freezing thoracic fluid and heart blood on filter papers from dead wildlife may significantly underestimate seroprevalence of *T. gondii*. If northern researchers and trained community monitors can perform this test in the field, this could have significant implications for food safety testing of hunted wildlife in the Canadian North. However, serum samples would be a better alternative than body fluids, which often contain particulate matter that might give false positives. Since a few samples demonstrated heterogeneous matter, especially fat in fluid from thawed

hearts, a chloroform clean-up method on all positive samples of frozen thoracic and heart fluids was performed. No antibodies against *T. gondii* were found after the procedure. It is possible that the chloroform extraction (used to address false positives due to lipid interference in samples from marine mammals) is less suitable for body fluids than sera and could lead to false negatives. Finally, all our estimates were lower than previous estimates of seroprevalence in geese from this region (39% in Ross' Geese and 36% in Lesser Snow Geese), suggesting that this MAT on thoracic and heart fluids may not be the best method of estimating exposure to *T. gondii* in these species. We did consistently find higher seroprevalence in Ross' vs Lesser Snow Geese, similar to previous studies, which may be linked to differences in diet, foraging behaviour, and differential exposure on staging and overwintering grounds.

We also compared 2 methods of extraction of DNA of *T. gondii* from pooled brain and heart of shot geese (magnetic capture vs a commercial kit), followed by PCR targeting a 529 bp repeating segment specific to *T. gondii*. No DNA of *T. gondii* was detected in heart and brain using either method of DNA extraction. These results suggest the possibility of a low infection status in Ross's and Lesser Snow Goose population at Karrak Lake. However, previous studies indicate exposure to and presence of DNA of *T. gondii* in tissues of Karrak Lake geese, and we suspect that our negative findings instead reflect the detection limit of the PCR. Other molecular techniques (i.e., a real-time PCR with higher sensitivity) may provide great insight in this matter.

3.1 INTRODUCTION

Toxoplasma gondii has been isolated and serologically detected in a vast array of wild avian species from all around the world (Dubey, 2002). Mortality has been reported in heavily infected birds as well (Dubey et al., 2001; Work et al., 2002). Despite the absence of free-ranging felid definitive hosts in the Arctic, *T. gondii* has been found in resident predators such as Arctic foxes (Sorensen et al., 2005; Elmore et al., 2016b), suggesting that the parasite enters this ecosystem via migratory animals, including Arctic-nesting geese. In fact, multiple studies have detected the organism in migratory birds acting as vectors in terrestrial Arctic ecosystems (Prestrud et al., 2007; Sandstrom et al., 2013), including the Arctic tundra of Karrak Lake, Nunavut (Elmore et al., 2014; Elmore et al., 2015). Prestrud et al. (2007) demonstrated a seropositivity of 7% in migratory barnacle geese on the high Arctic archipelago of Svalbard, whereas no resident herbivores such as reindeer and sibling voles had antibodies against the parasite. The same scenario occurred at Karrak Lake, with 39% seropositivity for Ross's Geese and 36% for Lesser Snow Geese (migratory herbivores), but no antibodies or DNA of *T. gondii* were detected in nonmigratory herbivores such as brown and collared lemmings (Elmore et al., 2015). Being herbivorous, geese likely become infected via consuming oocysts shed by Felidae into the environment while migrating or overwintering in temperate regions. As intermediate hosts, the parasite reproduces asexually and forms tissue cysts in their organs and muscles (Dubey, 2010b).

In the present study, the efficiency of a new commercially available modified agglutination test (MAT) for detection of *T. gondii* antibodies in thoracic and heart fluids of Ross's and Lesser Snow Geese was compared in field and laboratory conditions. MAT is a very useful serological technique that has been widely used in wildlife to assess exposure of a population (Dubey, 2010b). This direct agglutination assay is rapid and simple to perform, and doesn't require the use of complex laboratory facilities or species-specific conjugates. However, as for all serological tests, it indicates previous exposure, but not current infection status (Macri et al., 2009). A combination of serological and molecular tests is often

necessary to establish if an individual is really infected and whether it has been infected in the distant past or more recently. Even though previous versions of MAT have been described as highly sensitive and specific (Dubey et al., 2005; Hollings et al., 2013), there is a lack of validation for wildlife, and performance can differ among species and between tests (Macri et al., 2009; Zhu et al., 2012). Moreover, as cut-off values are based on visual inspection, observer bias in results can happen. Nevertheless, being able to perform the MAT in a remote region can have important impacts for public health in northern communities, by screening hunted wildlife and determining potentially contaminated meat for human consumption.

In the current study, four different types of goose samples were tested with the MAT kit: fresh thoracic fluid (processed in the field), frozen thoracic fluid, blood collected in the laboratory from frozen hearts, and thoracic fluid collected on filter paper. The last three reflect samples commonly collected from dead wildlife where fresh blood samples are unavailable, especially in remote regions where there may be long delays in shipping and processing. Filter paper blood collection is a simple, inexpensive and practical way of collecting blood (Curry et al., 2011; Curry et al., 2014). It has been used in a variety of serological studies around the world and is especially useful in remote areas (Andriamandimby et al., 2013; Aston et al., 2014). Use in wild waterfowl has also been described (Kraus et al., 2011; Elmore et al., 2014). Thoracic fluids from carcasses are easily obtained and can serve as alternative material to serum for analysis. Several studies successfully demonstrated presence of *T. gondii* antibodies in body fluids from many species (Lunden et al., 2002; Hamilton et al., 2005; Prestrud et al., 2007; Jakubek et al., 2012). However, antibody levels in fluid samples are usually lower than those in serum (Jakubek et al., 2012). One study showed that fluid samples from heart gave the strongest positive correlation when comparing *T. gondii*-specific antibody titers in meat juice of different tissue samples and serum (Wallander et al., 2015), although a commercial enzyme-linked immunosorbent assay (ELISA) kit was used instead of MAT. Another study did a similar experiment comparing both ELISA and MAT on serum

vs muscle fluids and found that both assays would be good candidates to test tissue fluids (Forbes et al., 2012). Since the goose heart samples and thoracic fluid were often contaminated with fat particles, which can interfere with reading the MAT, a chloroform/centrifugation method (Blanchet et al., 2014) was performed to remove fat.

We complemented these serological methods with molecular methods of detection, using both a commercially available DNA extraction kit and a novel magnetic capture (MC) method of extracting DNA from pooled heart and brain from three individual geese (pooled in order to reach the minimum amount required for the test and to increase detection probability). In experimentally infected geese, heart and brain had the highest probability of detection compared to other organs (Elmore et al., 2016a). By pooling heart and brain using magnetic capture, we can use up to 100g of tissues, compared to ≤ 25 mg by traditional DNA extraction, thus increasing the chance to find DNA of tissue cysts of *T. gondii*. The number of *T. gondii* tissue cysts can be very low, with as few as one tissue cyst present in 100 g of meat. Therefore, without using a concentration method (like magnetic capture), it is not practical to detect this low level of *T. gondii* infection (Hill and Dubey, 2002; Opsteegh et al., 2010). Magnetic capture is a fairly novel technique, and has been mostly used to screen domestic animals, most of them experimentally infected (Opsteegh et al., 2010; Jurankova et al., 2013; Jurankova et al., 2014a; Jurankova et al., 2015; Koethe et al., 2015). Its use in naturally infected wildlife has not been widely described.

As more animals are exposed than actively infected in a population, we hypothesized a lower detection using molecular than serological methods, but a higher detection for magnetic capture than traditional DNA extraction in goose tissues. We also hypothesized a higher prevalence using MAT in laboratory conditions than in the field, since the equipment available is often more reliable and accurate in the laboratory. Contamination of samples is also less likely to occur. Finally, using different sample types

(i.e., body fluids, filter paper, and frozen heart blood), that reflect the reality of wildlife studies in remote regions, can help inform further efforts to determine the significance of Arctic nesting geese as sources of *T. gondii* in northern ecosystems, where they serve as food sources for northern communities, as well as wildlife such as Arctic canids.

3.2 OBJECTIVES

- To determine presence of *T. gondii* in wild Arctic-nesting geese in the Karrak Lake ecosystem by comparing molecular and serological methods.
- To compare results for different samples from wild Arctic-nesting geese using a serological method (a new commercially available MAT) in the field versus laboratory
- To compare traditional vs magnetic capture PCR for detection of DNA of *T. gondii* in heart and brain of wild geese.

3.3 METHODS

3.3.1 Study area

The field site was at the large goose colony at Karrak Lake (67°14' N, 100°15' W) in the Queen Maud Gulf Bird Sanctuary, Nunavut, Canada, from May to July in 2014 and 2015. This Arctic goose colony consists of over one million nesting Ross's and Lesser Snow Geese each year, being one of the largest on Earth (Kerbes et al., 2006; Alisauskas et al., 2012; Kerbes et al., 2014). Geese usually arrive in late May and depart shortly after hatch in early July, dispersing throughout the colony. They migrate south in late August (Samelius et al., 2007; Alisauskas et al., 2011). Karrak Lake region consists of rolling tundra, dominated by rock outcrops, sedge meadows, and marshy areas interrupted by shallow tundra ponds (Ryder, 1972).

3.3.2 Field sample collection

Since 1991, an ongoing study of the population ecology of arctic nesting waterfowl has occurred annually on this site. Ross's Geese and Lesser Snow Geese were collected via shooting from late May to early July for field seasons in 2014 and 2015, and dissected on site. In between each carcass, gloves and scalpel blades were changed. Fluids from the thoracic cavity were collected on a single Nobuto filter paper strip (Advantec MFS, Inc, Dublin, CA, USA) for each goose. Filter strips were dried, stored in individual envelopes and kept at -20°C for further analysis at the University of Saskatchewan.

Temperature of the generator-powered freezer in the field may have experienced fluctuations since the power was sometimes switched off. During transport, filter strips were kept at room temperature, and refrozen at -20° C at the University of Saskatchewan. Thoracic fluids from carcasses, when available, were collected in 0.6ml Eppendorf microcentrifuge tubes (Fisher Scientific, USA) and frozen at -20°C. Whole heart and brain were collected as well and kept frozen at -20°. All samples were kept for about two months in the field freezer prior to shipping. Only samples from 2015 (Ross's n=32, Lesser Snow n=29) were analyzed and compared via serological and molecular methods. Samples from 2014 consisting of heart and brain (n=100) were used for magnetic capture trials since it is a novel technique in wildlife and validation needed to be performed for these species (Table 4).

All procedures were approved by the University of Saskatchewan Animal Care Committee (UCACS protocol number 20100159-19990029) and followed Guidelines of the Canadian Council on Animal Care. Goose samples were also collected under research permits from the Nunavut Department of Environment 2014-027/2015-017 and Canadian Wildlife Service NUN-MBS-14-02, NUN-SCI-14-02.

3.3.3 Filter Paper Elution

Manufacturer instructions and the method used by Curry et al. (2011) were followed. The filter paper was cut into small pieces and placed in a 1.5ml Eppendorf microcentrifuge tube. 1ml of Dulbecco's Phosphate Buffered Saline with antibiotic was added to the tube (resulting in a 1:25 dilution) and left at 4°C overnight. In order to eliminate any particles in suspension from dried blood clots, we filtered all

eluate samples through 19-gauge filter needle (Becton Dickenson Canada, Inc., Mississauga, ON) and transferred the filtrate into a new microcentrifuge tube (Elmore et al., 2014). The samples were tested immediately.

3.3.4 Serological Analysis: MAT in the field and in laboratory

A new commercially available modified agglutination test kit (New Life Diagnostic LLC, Carlsbad, CA, United States) (Al-Adhami et al., 2016) was used according to the manufacturer instructions. Since no refrigerator was available in the field, the kit was stored on the ground in the main field camp building, the only place with no freezing temperatures in May-June, for about a month prior to use. The temperature recorded on the ground was 11°C with some fluctuations throughout the day.

After centrifugation of freshly collected body fluids at 8000 rpm for 20 minutes, four dilutions were performed on goose body fluid supernatant (1:25-1:50-1:100-1:200). On a U bottom 96 well assay plate, 25µl of antigen mixture was mixed with 25µl of body fluid dilutions and incubated overnight. A blue button at the bottom of the well indicated a negative result. A clear bottom with agglutination covering 50-100% of the well at 1:25 dilution was considered positive. These procedures were also performed on thawed thoracic fluid in the laboratory at the University of Saskatchewan in order to compare with field results. After having been stored for 4 months in a -20°C freezer following fieldwork, the samples were thawed for 24h at 4°C and centrifuged at 1000 x g for 5min at 4°C before performing the test. In the laboratory, kits were stored in a refrigerator as per manufacturer's instructions.

After having been stored for 10 months in the laboratory at -20°C following fieldwork, hearts were thawed for 24h at 4°C and serosanguineous fluid collected in 1.5 ml Eppendorf microcentrifuge tubes. We centrifuged each sample at 1000 x g for 5 min at 4°C to remove debris (Hamilton et al., 2015). The supernatant was assayed immediately by MAT at a dilution of 1:40 and samples that showed agglutination covering 50-100% of the well were interpreted as positive. Questionable results consisting

of mild agglutination covering less than half of the test well were considered negatives. Samples containing too many particles seen with the naked eye were not included in the analysis.

3.3.5 Chloroform extraction method

All thawed heart fluid samples (regardless of serostatus) and positive samples from thoracic fluids were cleaned up via a chloroform/centrifugation method (Blanchet et al., 2014) to remove lipids and other particles. A 1ml sample was mixed with 1ml of chloroform and centrifuged at 1000 x g for 30 min. The upper phase was pipetted, transferred into another Eppendorf tube, and centrifuged once more (10 000 x g, 30 min). The supernatant was assayed by MAT at a dilution of 1:40, and compared with the results prior to chloroform treatment. A serum sample from a reindeer experimentally infected with *T. gondii* was used as a positive control.

3.3.6 Magnetic Capture

Magnetic capture was performed following Opsteegh et al. (2010) protocol. In each run of the protocol, a negative sample and two spiked samples (positive controls) were included. The concentration of the undiluted tachyzoite-stock used for spiking was 2.5×10^6 /ml. A 10-times dilution series was made in ultrapure water to obtain 2.5×10^4 and 2.5×10^5 /ml. 100µl of these dilutions was added to 50g of beef meat samples, resulting in samples spiked with 2500 and 25000 tachyzoites. Beef meat juice was tested by MAT to ensure that it was negative for *T. gondii*, beforehand.

Preparation of crude DNA extract:

A maximum of 100g of tissue (goose brain and heart pooled together for 3 individuals to reach minimum required + beef controls) was cut into 1cm³ pieces, put in a Stomacher bag with filter, and cell lysis buffer containing 100 mM Tris HCl pH 8.0, 5 mM EDTA pH 8.0, 0.2% SDS, 200 mM NaCl, 20 mg/ml proteinase K was added at 2.5 ml per gram of sample. Samples in bags were homogenized in a

Stomacher® 400 Circulator (Seward) for 2 min at high speed, and incubated at 55°C in a waterbath overnight. After incubation, 50 ml of homogenate was transferred to a 50 ml tube, and centrifuged for 45 min at 3500xg. Twelve millilitres of supernatant (crude extract) was transferred to a 15-ml polypropylene tube.

Removal of free biotin:

The crude extract was incubated at 100°C for 10 min to inactivate the proteinase K. After cooling down, washed streptavidine–sepharose (GE Healthcare, Bio-Science) was added. Samples were incubated for 45 min at room temperature, while rotating at 10rpm. Afterwards, centrifugation was performed for 15 min at 3500xg, and 10 ml of biotin-free supernatant was transferred to a 15-ml polypropylene tube.

Sequence-specific magnetic capture:

One picomole of 10µM of Tox-CapF and Tox-CapR were added to each supernatant. These Tox-oligonucleotides are complementary to the 529-bp repeat element (Homan et al., 2000). The supernatants were heated at 95 °C for 15 min to denature all DNA. The tubes were then transferred in a 55°C water bath, and left to allow for hybridization between capture oligonucleotides and *T. gondii* DNA for 45 min. The tubes were left to cool down to room temperature while rotating at 10 rpm for 15 min. Per sample, 80 µl of M-270 Streptavidin Dynabeads (Invitrogen, 10mg/ml) was washed in Binding & Washing (B&W) buffer (5 mM Tris HCl pH 7.5, 0.5 mM EDTA pH 8.0, 1 M NaCl) according to the manufacturer's instructions. The washed beads and 2 ml of 5 M NaCl were added to each supernatant sample and the samples were incubated rotating (10 rpm) at room temperature for 60 min. The tube was placed in a magnet (PolyAtract System1000, Promega) for 10 min and supernatant was removed. The beads were washed twice in B&W buffer, and resuspended in 50 µl of distilled water in a 1.5-ml tube. The bead suspension was heated at 100 °C for 10 min to release *T. gondii* DNA from the beads. The tube was placed in a magnet (DynaMag2, Life technologies) and the supernatant was immediately transferred to a clean 1.5-ml tube.

Finally, a trial on whole heart and geese brain pooled together and spiked with different tachyzoite concentrations was done to determine the lowest detection of *T. gondii* in such samples assayed by magnetic capture. Four replicates of three dilutions (2.5×10^5 , 10^6 , and 10^7 /ml) on 50 g of goose samples were assayed. Each sample consists of heart and brain of 3 individuals. A similar trial was performed on beef samples at the same dilution to seek the lowest detection limit of the PCR (Homan et al., 2000).

Table 4. Goose trial using heart/brain of 3 individuals pooled together for magnetic capture

Sample number	Animal Species	Tissue type	Grams of total tissue	Tachyzoites dilution added to samples
G1	Snow Geese (3)	Heart+Brain	50	2.5×10^5
G2	Snow Geese (3)	Heart+Brain	50	2.5×10^5
G3	Ross Geese (3)	Heart+Brain	50	2.5×10^5
G4	Ross Geese (3)	Heart+Brain	50	2.5×10^5
G5	Snow Geese (3)	Heart+Brain	50	2.5×10^6
G6	Snow Geese (3)	Heart+Brain	50	2.5×10^6
G7	Ross Geese (3)	Heart+Brain	50	2.5×10^6
G8	Ross Geese (3)	Heart+Brain	50	2.5×10^6
G9	Snow Geese (3)	Heart+Brain	50	2.5×10^7
G10	Snow Geese (3)	Heart+Brain	50	2.5×10^7
G11	Ross Geese (3)	Heart+Brain	50	2.5×10^7
G12	Ross Geese (3)	Heart+Brain	50	2.5×10^7
G13	Beef	Muscle	50	2.5×10^5
G14	Beef	Muscle	50	2.5×10^6
G15	Beef	Muscle	50	2.5×10^7
G16	Beef	Muscle	50	0

3.3.7 Traditional DNA extraction

DNA extractions were performed on 25mg of brain and heart pooled together for each individual goose. Each sample was frozen at -80°C for 30min and thawed in a 97°C waterbath for 15min, repeated twice. Afterwards, samples were analysed via DNeasy® Blood and Tissue Kit (Qiagen, Toronto, Canada) according to the manufacturer instructions.

3.3.8 PCR Amplification

A conventional PCR using the primers TOX4 and TOX5 described by Homan et al. (2000) which amplify a 200-to 300-fold repetitive 529-bp repeat element within the *T. gondii* genome was performed on each DNA sample from both DNA extraction techniques. The optimized amplification was performed in a 25µl reaction mixture containing 2µl template DNA, 0.5µl of each primer (10 µM, Sigma Life Science), 1.25µl 10X PCR Buffer (500mM KCl, 200mM Tris-HCl, Invitrogen), 0.75µl MgCl₂ (1.5mM, Invitrogen), 0.5µl dNTP (10µM, Invitrogen), 0.2µl Taq DNA polymerase (0.5U, Invitrogen), and 19.3µl ultrapure water. Amplification was performed in an Eppendorf Mastercycler PCR machine with initial denaturation for 7 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 63°C and 1 min at 72°C, and a final extension for 10 min at 72°C. The sequence was visualized through electrophoresis in 1 % agarose gel and stained with ethidium bromide. Positive and negative controls were included in all PCR runs.

Optimization of the PCR conditions (hybridization temperature for primer annealing, MgCl₂, DNA Taq polymerase and primer concentrations) was performed using DNA of *T. gondii* from experimentally infected reindeer in order to enhance the sensitivity and specificity of the reaction (Chabbert et al., 2004; Bastien et al., 2008).

Finally, to avoid false-negative results, all negative samples were tested once more by PCR using primer sets that target the internal positive-control, the species-specific reference gene, to assess the quality of DNA extracted.

3.3.8.1 Post Amplification Analysis

When multiple amplifications were observed under ultraviolet light, samples that had suspect-positive bands of appropriate size for the primer set used were pooled together in order to increase the amount of DNA, and run on a small 1% agarose gel in one large well. The band showing a result close to the 529bp

element was excised and extracted using QIAquick® Gel Extraction. The purified products were sent for commercial DNA sequencing. The resulting sequences were subjected to nucleotide BLAST analysis.

3.3.9 Data Analysis

All tests were read by the same person, and performed at different times. The reader was blinded between assays. We estimated apparent seroprevalence by dividing the number of geese that were clearly *T. gondii* seropositive by the total number of samples tested. Statistical analyses were performed using IBM SPSS Statistics 20.

3.4 RESULTS

Antibodies were found in body fluids when MAT was performed in the field (Ross = 1/5 (20%, 95% CI = 0.00-0.76)), Snow = 2/16 (12.5%, 95% CI = 0.00-0.31)) and laboratory (Ross = 4/27 (14.8%, 95% CI = 0.00-0.29)), Snow = 2/21 (9.5%, 95% CI = 0.00-0.23)), as well as in serosanguineous fluid collected from frozen hearts (Ross = 1/32 (3.1%, 95% CI = 0.00-0.10)), Snow = 1/29 (3.4%, 95% CI = 0.00-0.11)). Of the three geese that tested positive in the field on fresh thoracic fluid, one was positive in the laboratory. One goose that tested negative in the field was positive when frozen thoracic fluid was tested in the laboratory. Regarding MAT on filter paper eluates, no antibodies were found for either species, although 20 samples showed a weak red color after dilution, indicating that extraction of antibodies had failed for these specific samples (see Table 5). No antibodies were seen after performing the chloroform/centrifugation method in body and heart fluids.

Table 5. MAT and results on goose samples collected at Karrak Lake in 2015

Geese ID	Fresh thoracic fluids tested in the field (n=21)				Frozen/thawed thoracic fluids tested in the lab (n=48)			Frozen/thawed heart fluids (n=61)	Thoracic fluids on filter paper dried and frozen (n=41)		
	1:25	1:50	1:100	1:200	1:25	1:50	1:100	1:40	1:25	1:50	1:100
ROGO01											
ROGO02									Extraction failed*		
ROGO03											
ROGO04											
ROGO05											
ROGO06	**										
ROGO07									Extraction failed		
ROGO08											
ROGO09									Extraction failed		
ROGO10											
ROGO11											
ROGO12									Extraction failed		
ROGO13											
ROGO14											
ROGO15									Extraction failed		
ROGO16											
ROGO17											
ROGO18											
ROGO19									Extraction failed		
ROGO20											
ROGO21											
ROGO22									Extraction failed		
ROGO23											
ROGO24									Extraction failed		
ROGO25											
ROGO26											
ROGO27									Extraction failed		
ROGO28											
ROGO29											
ROGO30											
ROGO31											
ROGO32											
SNGO01											
SNGO02											
SNGO03											
SNGO04									Extraction failed		
SNGO05											
SNGO06											
SNGO07											
SNGO08											
SNGO09									Extraction failed		
SNGO10											
SNGO11											
SNGO12											
SNGO13											
SNGO14											
SNGO15									Extraction failed		
SNGO16											
SNGO17											
SNGO18											
SNGO19									Extraction failed		
SNGO20											
SNGO21											
SNGO22									Extraction failed		
SNGO23											
SNGO24											
SNGO25											
SNGO26											
SNGO27											
SNGO28											
SNGO29											

*Extraction failed: sample showing weak red color after dilution, not included in analysis

**Green = positive results Red = negative results

Suspect-positive bands corresponding to the 529bp element were visualized with DNA extracted using the DNeasy® Blood and Tissue Kit in 18 Ross's (including all 5 that tested positives on fresh and frozen/thawed thoracic fluids) and 9 Lesser Snow Geese (including one that tested positive on fresh thoracic fluids). Gel electrophoresis showed that many of the PCR products produced with both primer sets were not of the appropriate size, which indicates non-specific amplification, thus the necessity to sequence the bands to confirm diagnosis. All the samples showing the same suspect band were pooled, isolated and sent for sequencing. The resulting sequence gave no match when subjected to nucleotide BLAST analysis. For DNA extracted using the magnetic capture method, suspect-positive bands were observed in 9 Ross's Goose samples and 6 Lesser Snow Geese (each sample includes 3 individuals). All seropositive geese were part of the suspect-positive bands except for one SNGO. All the samples showing the same suspect band were pooled, isolated and sent for sequencing. Again, *T. gondii* was not confirmed in any of the suspect-positive samples, although the sequence had 94% similarity to that of *Anas platyrhynchos* (commonly called Mallard).

Finally, our magnetic capture trial on 50 g of heart and brain pooled together at four replicates of three dilutions (2.5×10^5 , 10^6 , and 10^7 /ml) gave the following results:

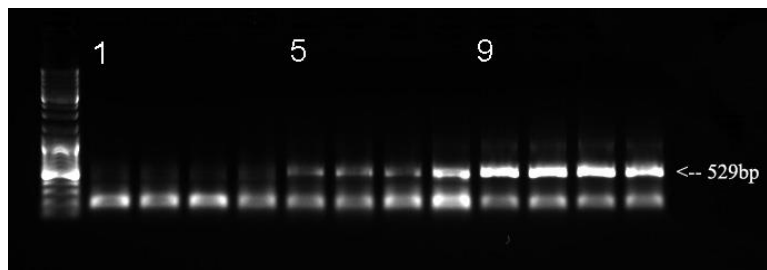


Figure 2. MC trial on heart/brain of geese given three different tachyzoite concentrations at four replicates

These results confirm a minimal detection of 2.5×10^5 *T. gondii* tachyzoites per ml (#1-4) using conventional PCR targeting the 529bp element. This amount corresponds to a minimal detection of 50000

tachyzoites per 100 gram of tissue. The same minimal detection was found in 50g of beef samples used as our controls.

3.5 DISCUSSION

Our results demonstrated exposure of *T. gondii* in the Ross's and Lesser Snow Goose population at Karrak Lake (20% and 12.5% respectively for thoracic fluids in the field; 14.8% and 9.5% respectively for frozen/thawed thoracic fluids in the lab; 3.1% and 3.4% respectively for frozen/thawed heart fluids; 0% and 0% respectively on blood on filter paper strips). A recent study (Elmore et al., 2014) demonstrated a seroprevalence on blood filter paper eluent using MAT and IFAT in 26% Ross's Geese and 25% Lesser Snow Geese in this ecosystem, which is higher than our serology findings. Another study found a seroprevalence of 32.4% in Ross's Geese and 28.3% in Lesser Snow Geese using serum samples (Elmore et al., 2015). Our small sample size and the type (body fluids vs sera) and quality of the samples could account for the lower prevalence in our study. Temperature fluctuations in the field freezer could have contributed to the degradation of samples. Concerning other arctic nesting goose populations, Prestrud et al. (2007) found a seroprevalence of 7% in Barnacle Geese from Svalbard, and 6.5% in Pink-Footed Geese in another study (Sandstrom et al., 2013), as well as 25% in migratory Barnacle Geese in the Netherlands. The modified agglutination test remains one of the most commonly used serological method for detection of anti-*T. gondii* antibodies (Dubey, 2010b), and many different cut-off values are used among studies (Dubey et al., 1995b). It thus makes the comparison between different species and regions quite difficult (Blanchet et al., 2014).

I performed the MAT test in the field for the first time, which is a new application for this commercial kit and suggests that northern researchers and trained community members can perform this test in the field instead of having to store and ship the samples for analysis by southern diagnostic

laboratories. This could have significant implications for food safety testing of hunted wildlife in the Canadian North, especially if the test can be used on meat juice. Our results, however, suggest that body fluids are less suitable than sera since the heterogeneity and quality of the samples appear to have affected the results. Serological methods have been used to screen and monitor animals destined for human consumption for *T. gondii* (Maksimov et al., 2011; Steinparzer et al., 2015). Because only a limited number of samples were available, no statistical analysis was performed. However, the results suggest that MAT on fresh thoracic fluids in the field provided the best serological results in comparison with filter paper strips, frozen thoracic fluids and frozen heart fluids in laboratory, although it is difficult to draw conclusion because of our small sample size. We would have expected a higher detection in laboratory by having access to better facility and resources. Long and suboptimal storage in a remote area, as well as multiple freeze/thaw cycles may therefore have led us to underestimate seroprevalence of *T. gondii* in our study. Finally, regarding the one sample negative on fresh thoracic fluid but positive on frozen thoracic fluids, the possibility of false-positive could have occurred. In all serological assays and especially with samples from wildlife species, risk of crossreactivity with unknown non-target antibodies is possible, which could lead to ambiguous and misclassified test results. Moreover, results from MAT are based on visual inspection, which can differ between different observers regarding their experience and opinion (Elmore et al., 2014).

In this work, no antibodies were found by MAT on filter paper elution. Only one filter paper was used per bird and we used a 1:25 dilution using one filter paper, whereas Elmore et al. (2014) performed a 1:20 dilution using two filter papers. Our high dilution and failure of extraction for many samples could explain the negative results. We did get positive results when using body fluids on MAT in the field and laboratory, as well as fluids from frozen heart. Mixture of blood and tissue fluids are often used instead of serum, and constitute a good alternative for serological surveys (Tryland et al., 2006; Glor et al., 2013), although such samples will often be heterogeneous by having particulate matter in suspension. When

tested, false positives might occur after non-specific adherence to tachyzoites in MAT. After a chloroform clean-up to remove lipids from heart samples and thoracic fluids, no antibodies were detected. One study on porpoises had similar outcomes when samples were assayed via MAT with and without chloroform clean-up (Blanchet et al., 2014). Chloroform had no adverse effect on antibody activity when used on egg yolk and human serum sample (Polson, 1990; Castro et al., 2000), but not on body and meat fluids. Antibody levels in fluid samples are usually lower than those in serum (Jakubek et al., 2012). Although possible that all our samples were truly negative, it is very unlikely that the Arctic nesting geese population at Karrak Lake has no exposure to *T. gondii*; positive serostatus has been demonstrated by Elmore et al. (2014, 2015), and DNA has been found in tissues (Elmore et al., 2016a). The quality at the time of testing, and type of samples could be the reason why no positive results were detected after the chloroform clean-up.

The use of PCR can determine if DNA of the parasite is present in tissues (a marker of infection) whereas serological methods cannot. The sensitivity and specificity of the PCR depend on multiple factors, such as the DNA extraction protocol, the characteristics of the DNA sequence that is amplified, and the optimization of the reaction conditions (Edvinsson et al., 2004). In fact, the TOX4 and TOX5 primers are not specific to *T. gondii*, and also amplify *N. caninum*, a coccidian parasite closely related to *Toxoplasma* (Elmore et al., 2015). We were not able to detect *T. gondii* DNA in our goose samples. We would have expected a higher detection via magnetic capture than traditional DNA extraction due to the greater amount of tissue in the MC extraction. Our negative results can indicate low infection rates in Ross's and Lesser Snow Geese. However, previous studies indicate exposure to and presence of DNA of *T. gondii* in tissues of Karrak Lake geese (Elmore et al., 2015; Elmore et al., 2016a). We suggest the use of a quantitative real-time PCR (qPCR) targeting the 529bp element instead of conventional PCR. Quantitative real-time PCR is generally considered to be more sensitive. In fact, Opsteegh et al. (2010) used a qPCR targeting the 529bp element along with magnetic capture, and was able to detect 230

tachyzoites per 100 g of meat, compared to a minimal detection of 50000 tachyzoites per 100 g of meat in our trial with magnetic capture followed by conventional PCR. Since infected animals often carry tissue cysts containing bradyzoites instead of tachyzoites, the number of tissue cysts cannot be estimated. One tissue cyst may contain only two bradyzoites, while others can contain more than thousand of organisms (Dubey et al., 1998). With our detection limit estimated at 50000 free tachyzoites per 100 g of meat, one cyst containing hundreds of bradyzoites might be detectable. However, we also could easily miss an animal infected at a lower degree regarding the possibility of a low number of bradyzoites. With Opsteegh et al. (2010) detection limit of 230 parasites per 100 g, one cyst would be detectable in most cases. One recent study in brain tissue of wild mice demonstrated that magnetic capture followed by qPCR targeting the 529bp element of *T. gondii* was more sensitive than commercial tissue kit/conventional PCR (Jurankova et al., 2014b), with a prevalence of 1.2% and 0% on 243 individuals, respectively, and a detection of 1-5 tachyzoites by qPCR following magnetic capture.

In summary, antibodies to *T. gondii* were detected in body and heart fluids in Ross's and Lesser Snow Geese at various dilution levels by MAT. Even though no antibodies were detected after performing the chloroform/centrifugation method, the quality of samples (multiple frozen/thawed cycles when tested) as well as the type (body fluids instead of serum) could explain the negative results. Exposure in Karrak Lake geese has been reported before, by using both filter papers and serum (Elmore et al., 2014; Elmore et al., 2015). DNA of *T. gondii* was not found in heart and brain of both species using magnetic capture and traditional extraction followed by conventional PCR, likely as a result of the low detection limit of the latter; we suggest the use of alternative methods (i.e., magnetic capture followed by qPCR) for further research. Lesser Snow Geese and Ross's Geese are commonly hunted throughout their northern and southern range. The demonstration of infection, not only exposure, is necessary to assess the risk of transmission to predators (i.e., Arctic foxes) as well as hunters and people handling carcasses. Finally, we successfully demonstrated the use of MAT in the field which can help screen for potential contaminated

wildlife meat destined for human consumption in remote northern communities. However, further validation is needed to confirm the results since false-negatives might occur using body fluids instead of serum.

CHAPTER 4: Experimental infection of reindeer

Transition Statement

In Chapter 3, we demonstrated exposure to *Toxoplasma gondii* in the Ross's and Lesser Snow Goose population at Karrak Lake, a migratory species that could be exposed to oocysts on their migration route or overwintering grounds. In Chapter 2, I demonstrated that Arctic foxes are exposed to the parasite, and could become infected by scavenging on geese and other wildlife, such as caribou. As for the geese, caribou are a migratory species and their meat is an important food source for Northern communities and wild carnivores. Studies of caribou in the Canadian North have shown that they are exposed to *T. gondii*. Caribou populations are declining throughout the Arctic, and therefore it is not possible to obtain samples from harvested animals. In this chapter, I seek to determine effects of *T. gondii* on the health of experimentally infected reindeer (conspecific with caribou), and the tissue distribution and pathology of the parasite. As a zoonotic agent, detection of *T. gondii* in tissues of animals destined for human consumption is a concern for food safety and public health. Determining how Arctic peoples become exposed and the health effect of toxoplasmosis in threatened wildlife is essential in order to implement culturally appropriate and effective local prevention measures.

Abstract

Toxoplasma gondii is a zoonotic parasite found worldwide including the Inuit in Canada, who are thought to be exposed through handling and consumption of Arctic wildlife. Caribou are historically the most important wildlife species harvested for food across the Canadian North, and other studies have shown that they are exposed to *T. gondii*. Since caribou populations are declining, we did not have access to samples from naturally infected caribou. Therefore, we experimentally infected reindeer (same species as caribou) to assess clinical signs of infection, pathology, and distribution of *Toxoplasma* in different tissue samples, especially muscles intended for human consumption. Three captive reindeer were infected via stomach intubation with three different doses of oocysts (1000, 5000, 10000); one was kept as an uninfected control. Reindeer were monitored daily for any behavioral changes, and signs of stress, aggressiveness and depression were noted for only the first two weeks following infection. Using a modified agglutination test (MAT), serostatus was negative prior to infection and all infected reindeer seroconverted four weeks post-infection. At 20 weeks post infection, reindeer were euthanized, and no gross abnormalities were observed on necropsy. Histopathology and immunohistochemistry were performed on heart, liver, spleen, lung, kidney, brain, tongue, diaphragm, triceps, external oblique, gluteus medius, and longissimus of the infected reindeer. *Toxoplasma gondii* cysts were visualized in tongue, diaphragm, external oblique, and triceps of the reindeer given the highest dose of oocysts, and on external oblique and gluteus medius in the reindeer given the lowest infective dose. Focal pleuritis and alveolitis were visualised on histopathology in this animal. No evidence that it was linked to *Toxoplasma* was found microscopically, but respiratory problems were noted. This reindeer expressed breathing difficulties and excessive panting for a week following infection. One brain cyst was also detected by microscopy of brain squashes at 60X directly after necropsy on the animal given the middle dose. The uninfected control reindeer (examined at 7 days pre-infection) remained seronegative and no changes were detected on histopathology (heart, liver, spleen, lung, kidney, rumen, and brain). Immunohistochemistry was not performed on the control. In addition to microscopy and

immunohistochemistry, two DNA extraction methods followed by conventional PCR targeting a 529bp repeating element specific to *T. gondii* were performed on tissue of all four reindeer. DNA of *T. gondii* was detected following traditional extraction (using a commercial kit that uses 25 mg of tissue) on a few muscles and organs (longissimus, gluteus biceps, heart, tongue, brain) and by magnetic capture on multiple tissue samples (longissimus, gluteus medius, gluteus biceps, external oblique, triceps, diaphragm, tongue, brain, heart, brain, and lung). Magnetic capture DNA recovery resulted in higher detection of *T. gondii* DNA and we suggest the use of this technique, which allows examination of large amounts of tissue (100g), for future food safety investigations.

This research provides important information about how Arctic peoples become exposed and the health effects of toxoplasmosis in wildlife, especially concerning migratory caribou, since the population is currently declining throughout the Arctic. With the observation of subtle behavior changes following infection in combination with the stress of climate and anthropogenic changes, *T. gondii* may have detrimental effects in health of wild caribou populations. Furthermore, the presence of *T. gondii* in all tissues tested commonly consumed raw, smoked, or dried in northern communities' raises concern regarding the safety of caribou meat as food source in naturally infected animals. This work highlights the need for more information on the significance of food-borne routes of transmission of *T. gondii* in the North, essential for implementing culturally appropriate and effective local prevention measures in northern residents.

4.1 INTRODUCTION

Caribou and reindeer herds are declining globally across their circumpolar range (Vors and Boyce, 2009; Weckworth et al., 2012). In addition to the increase of temperature and precipitation, anthropogenic landscape change and habitat alterations by industrial activities place caribou health and population viability at risk (Joly et al., 2015). Caribou are an inextricable part of the North, and play a crucial role in culture and survival for northern people; for generations, they were the most abundant cervid in the country (Festa-Bianchet et al., 2011). They also are a keystone species supporting many predator populations in northern Canada, including wolves, grizzly bears and Arctic foxes (Mowat and Heard, 2006; Musiani et al., 2007; Samelius et al., 2007). Although *Rangifer* populations historically fluctuated (Klein, 1991; Gunn, 2003), the current decline concurrent with global warming emphasizes the species vulnerability to these changes, and the potential to irrevocably alter the factors driving the population dynamics (Vors and Boyce, 2009). The distribution and abundance of predators, alternate prey, and parasites or diseases may also be affected by climate change, thus affecting caribou populations.

In Canada, the current distribution of caribou spreads across boreal, montane, and arctic ecozones in most Provinces and all Territories. Within their distribution range, caribou exhibit variability in ecology, genetics, behaviour and morphology, making their conservation and management particularly complicated (COSEWIC, 2011). Industrial development is increasing in the Arctic, and is impacting populations of caribou. Population impacts have been documented in boreal caribou, but barren-ground caribou herds may be declining as well (Festa-Bianchet et al., 2011; Joly et al., 2015). Loss of caribou will significantly and negatively affect socioeconomics for northern indigenous communities. For example, the replacement value of the annual harvest of the Beverly and Qamanirjuaq caribou herds, occupying mainly southeastern NWT, northeastern Saskatchewan, southern Nunavut, and northern Manitoba, was valued at more than \$20 million in 2008 (revised in 2013) (InterGroupConsultants, 2008).

Besides economic values, caribou remains the most important terrestrial subsistence resource for northern aboriginal peoples (Kendrick et al., 2005).

The apicomplexan parasite, *Toxoplasma gondii*, has a worldwide distribution and can be found in virtually all warm-blooded animals, including wildlife, domestic animals, and humans. It is widespread among humans and its prevalence varies widely from place to place (Dubey, 2009d). This ubiquitous parasite can cause several clinical syndromes including encephalitis, chorioretinitis and congenital infection (Kim and Weiss, 2008), and are not only limited to humans. Prevention of infection in its animal hosts is important for food safety, public health, and welfare aspect (McDonald et al., 1990; Dubey, 2009c, b; Kijlstra and Jongert, 2009). Many animal species utilized for food or fur have been found seropositive for *T. gondii* in the Canadian Arctic, including caribou, bears, muskoxen, geese, wolverines, and seals (Kutz et al., 2000; Kutz et al., 2001; Measures et al., 2004; Dubey et al., 2008; Reichard et al., 2008; Elmore et al., 2015). Kutz et al. (2001) found a higher seroprevalence in mainland caribou than island caribou, probably due to their migration range below the tree line where free-ranging felids (such as *Lynx canadensis*) shedding oocysts can be found. Ungulates in Alaska also demonstrated seropositivity to *T. gondii*, including moose, Dall sheep, and caribou (Zarnke et al., 2000; Stieve et al., 2010). In other part of the world, serologic evidence has been found in reindeer (conspecific with caribou) from Finland and Norway, correlated with age and feeding management style (Oksanen et al., 1997). However, serosurveillance in natural populations can only tell us if animals have been exposed, and not if the animals are actively infected or if they are experiencing negative health consequences of infection. The only report of toxoplasmosis in experimentally infected reindeer has been described in Fennoscandia, resulting in acute enteritis a few days after intraruminal inoculation with *T. gondii* oocysts (Oksanen et al., 1996). Finally, transplacental toxoplasmosis has been documented in a full term stillborn reindeer in a private farm in Texas, demonstrating vertical transmission leading to fatal toxoplasmosis (Dubey et al.,

2002). Other than these two studies, the clinical and pathological effects of *T. gondii* on *Rangifer* spp. are largely unknown, as is the potential for this parasite to play a role in population declines.

Certain zoonotic parasitoses are relatively common in people in Arctic communities, including *T. gondii* in the Inuit in Nunavut and Nunavik, Canada, who have a seroprevalence 2-5 times higher than the North American average of ~15% (Jones et al., 2008; Messier et al., 2009). In Nunavik, toxoplasmosis was strongly associated with consumption of raw caribou, seal meat and skinning of animals for fur (McDonald et al., 1990; Messier et al., 2009). In Kuujjuarapik, Nunavik, where Cree and Inuit populations live together, Messier et al. (2009) found much lower seroprevalence in Cree populations, who usually cook meat thoroughly, whereas Inuit often eat their meat raw, frozen or dried (especially seal, beluga, walrus, fish, and caribou). It is not known which organs or muscles contain *T. gondii* cysts that can be infective for humans and animals depending on this valuable source of food.

Therefore, we sought to determine the effects (clinical and pathological) and tissue distribution of *T. gondii* in experimentally infected reindeer (conspecific with caribou). In our study, we compared two molecular techniques to detect *T. gondii* DNA in tissue. We hypothesized a higher detection via magnetic capture (which allows testing of 100g of tissue) than traditional DNA extraction (using a commercial kit which tests 25mg of tissue). Although increasingly applied for food safety testing in Europe, magnetic capture (MC) DNA extraction methods have not been widely used in wildlife yet, especially with the use of conventional PCR instead of quantitative real-time PCR (Opsteegh et al., 2010; Jurankova et al., 2013; Jurankova et al., 2014a; Jurankova et al., 2015; Koethe et al., 2015). The comparison of both techniques in experimentally infected wildlife will give us information regarding sensitivity of a conventional PCR targeting the 529bp repeat element (Homan et al., 2000) when used with MC technique instead of traditional DNA extraction. We also hypothesized a higher detection of the parasite in brain, heart and skeletal muscles, as has been described in the literature (Jurankova et al., 2014a; Jurankova et al., 2015; Koethe et al., 2015).

4.2 OBJECTIVES

- To determine clinical effects and pathology of *T. gondii* in experimentally infected reindeer.
- To determine *T. gondii* tissue predilection in experimentally infected reindeer.
- To compare traditional vs magnetic capture methods for detection of *T. gondii* DNA in tissues of experimentally infected reindeer.

4.3 METHODS

4.3.1 *T. gondii* oocysts

In collaboration with the Centre for Food and Animal Parasitology (CFAP, Saskatoon, SK), a freshly generated batch of sporulated *T. gondii* oocysts of the VEG strain were obtained from cats. The isolate provided was type III, mildly virulent to mice. After collection of cat feces, fecal floats were incubated in 2% sulfuric acid at room temperature for one week to allow sporulation of oocysts. Afterwards, they were stored for 2 months in 2% sulfuric acid at 4°C prior to infection. A few hours before the inoculation, they were washed free of acid and suspended in saline. Three dilutions were made in saline at 10000, 5000 and 1000 oocysts in 5 ml. These dilutions were chosen in order to establish subclinical infections and avoid severe health problems.

4.3.2 Animals

Three reindeer (*Rangifer tarandus*) males of three years, surplus from the University of Calgary captive herd, were transported and housed outdoors for 4 months prior to infection at the Alternative Livestock Facility (ALF), part of the Western College of Veterinary Medicine (WCVN) at the University

of Saskatchewan. One reindeer male of 3 years old already housed at the ALF was also used to increase the sample size of our study. Sera were analyzed via modified agglutination test (MAT) at 1:25 dilution to confirm negative serostatus prior to infection. Reindeer were fed a mix of alfalfa/hay, beet pulp, and a CO-OP@ BUCK (DEER) RATION (Federated Co-Operatives Limited, Saskatoon, SK) supplemented in selenium. No coccidiostats were added to the feed. Salt blocks and water were available ad libitum. At inoculation, reindeer weight was estimated at approximately 150kg.

All procedures were approved by the University of Saskatchewan Animal Care Committee (UCACS protocol number 20130107) and follow the Guidelines of the Canadian Council on Animal Care.

4.3.3 Reindeer anesthesia protocol

Zuclopenthixol acetate (Clompixol® Depot, 200mg/ml, 1mg/kg, Lundbeck) was administered for transport and movement. For manipulations (i.e., de-antlering, blood sampling, inoculation, and euthanasia), a combination of medetomidine hydrochloride (Domitor®, 40mg/ml, 0.12mg/kg) and ketamine hydrochloride (Vetalar®, 100mg/ml, 2mg/kg) was administered via darting. Atipamezole hydrochloride (Antisedan®, 5mg/ml, 0.2mg/kg, Pfizer) was used to reverse sedation.

4.3.4 Experimental design

Three reindeer were transported to the Vaccine and Infectious Disease Organization (VIDO), Saskatoon, SK, in level 2 indoor facilities a few hours prior to infection. They were inoculated via nasogastric tubes (.05" ID) with *T. gondii* oocysts suspended in water at a dose of 1000, 5000, and 10000 oocysts. They were kept under observation and transported back to the ALF 72 hours post infection, where they were housed in a small pen for the first week in order to monitor them closely. Ten days after infection, they were moved to a large pasture of six acres. They were observed daily for the first week and

every 2-3 days afterwards to assess any clinical signs, behavioral changes, and food intake. Blood samples were collected at 3 days, 4 weeks, and 20 weeks post infection, at the moment of euthanasia. A fourth, negative control reindeer was kept at the Alternative Livestock Facility. This animal died of unrelated causes and tissues were harvested at 7 days pre-infection.

4.3.5 Euthanasia and necropsies

Reindeer were heavily sedated and euthanized in the field by intravenous barbiturate overdose of sodium pentobarbital (Euthanyl-Forte®, 540mg/ml, MTC). They were immediately transported to the WCVL where necropsies were performed. The carcasses were examined for gross abnormalities, and organs and tissues collected (brain, heart, lung, liver, spleen, kidneys, eyes, tongue, diaphragm, and 17 different skeletal muscles). Fresh brain squash smears were performed at the time of necropsies and observed by microscopy at 60X. A subset of tissue samples were fixed in 10% formalin for histology and immunohistochemistry. The rest of the samples were frozen at -20°C ranging between a few days to six months prior to molecular testing.

4.3.6 Histology and Immunohistochemistry on reindeer tissues

Formalin-fixed tissues from all reindeer (brain, heart, lung, liver, spleen, kidneys, tongue, diaphragm, triceps, external oblique, gluteus medius, and longissimus) were processed into paraffin-embedded tissue blocks, sectioned and stained with hematoxylin and eosin (HE) for microscopic evaluation. For immunohistochemistry, 5-µm sections of the same paraffin-embedded blocks were cut and processed for IHC staining as previously described (Cruickshank et al., 1990) with some modifications. Briefly, epitope retrieval was performed using a proteinase K treatment and binding of the primary antibodies (rabbit anti-*Toxoplasma gondii* (1:100), ThermoFisher Scientific, Waltham, MA, USA) was detected using an avidin-biotin HRP-labelled detection reagent (Vector Labs, Burlingame, CA, USA) on an automated slide stainer

(Autostainer Plus, Dako Canada Inc., Mississauga, ON). IHC was conducted at Prairie Diagnostic Services, SK, Canada. All HE and immunostained sections were examined with a compound microscope.

4.3.7 Serology

Sera of all reindeer were tested for anti-*T. gondii* IgG using a commercially available modified agglutination test (MAT) (New Life Diagnostic LLC, Carlsbad, CA, United States) (Al-Adhami et al., 2016). The kit was used according to the manufacturer instructions, performing four dilutions (1:25-1:50-1:100-1:200).

4.3.8 Traditional DNA extraction (commercial kit)

DNA extractions were performed on 25mg of each of brain, heart, lung, diaphragm, triceps, external oblique, gluteus medius, biceps, and longissimus for each infected reindeer. Heart, tongue, and gluteus biceps of the unexposed control reindeer were also tested. Each sample was frozen at -80°C for 30min and thawed in a 97°C waterbath for 15min, repeated twice. Afterwards, samples were analysed via the DNeasy® Blood and Tissue Kit (Qiagen, Toronto, Canada) according to the manufacturer instructions.

4.3.9 Magnetic capture DNA extraction

Magnetic capture (MC) was performed on 100g of each tissue described above for each reindeer (excepted tongue for all reindeer and gluteus biceps for reindeer given 5000 oocysts) as per Opsteegh et al. (2010). For the uninfected reindeer, only gluteus biceps was tested with MC technique. In each run of the protocol, a negative sample and two spiked samples (positive controls) were included. The concentration of the undiluted tachyzoite-stock used for spiking was 2.5×10^6 / ml. A 10-times dilution series was made in ultrapure water to obtain 2.5×10^4 and 2.5×10^5 /ml. 100µl of these dilutions was added

to 100g of beef meat samples, resulting in samples spiked with 2500 and 25000 tachyzoites. Beef meat juice was tested by MAT to confirm negativity for antibodies to *T. gondii*.

4.3.10 PCR Amplification

A conventional PCR using the primers TOX4 and TOX5 described by Homan et al. (2000) which amplify a 200-to 300-fold repetitive 529-bp repeat element within the *T. gondii* genome was performed on each DNA sample from both DNA extraction techniques. The optimized amplification was performed in a 25µl reaction mixture containing 2µl template DNA, 0.5µl of each primer (10 µM, Sigma Life Science), 1.25µl 10X PCR Buffer (500mM KCl, 200mM Tris-HCl, Invitrogen), 0.75µl MgCl₂ (1.5mM, Invitrogen), 0.5µl dNTP (10µM, Invitrogen), 0.2µl Taq DNA polymerase (0.5U, Invotrogen), and 19.3µl ultrapure water. Amplification was performed in an Eppendorf Mastercycler PCR machine with initial denaturation for 7 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 63°C and 1 min at 72°C, and a final extension for 10 min at 72°C. The sequence was visualized through electrophoresis in 1 % agarose gel and stained with ethidium bromide. Positive and negative controls were included in all PCR runs.

Optimization of the PCR conditions (hybridization temperature for primer annealing, MgCl₂, DNA Taq polymerase and primer concentrations) was performed using *T. gondii* DNA extracted from experimentally infected reindeer in order to enhance the sensitivity and specificity of the reaction (Chabbert et al., 2004; Bastien et al., 2008). Finally, to avoid false-negative results, all negative samples were tested once more by PCR using primer sets that target the internal positive-control, the species-specific reference gene, to assess the quality of DNA extracted.

4.3.11 Post Amplification Analysis

Following gel observation under ultraviolet light, PCR samples that had suspect-positive bands of appropriate size for the primer set used were DNA purified using a QIAquick PCR Purification Kit (Qiagen, Toronto, Canada) and sent for commercial DNA sequencing. The resulting sequences were subjected to nucleotide BLAST analysis.

4.3.12 Data analysis

All tests were read by the same person, and performed at different times. The reader was blinded in between assays regarding the infection status of animals. Statistical analyses were performed using IBM SPSS Statistics 20. For serology, questionable results consisting of mild agglutination covering less than half of the test well were considered negatives.

Table 6. Experimental infection design on reindeer

Animal ID	Method of inoculation	Dose of oocysts given orally	Week of necropsy after infection	Post infection serology (MAT)				
				1:25	1:50	1:100	1:200	Week blood samples were taken
Reindeer control	-	-	-1	-	-	-	-	-1
Reindeer 1	Orally via nasogastric tube	1000	20	- + +	- + +	- + +	- + +	0 4 20
Reindeer 2		5000	20	- + +	- + +	- + +	- + +	0 4 20
Reindeer 3		10000	16	- + +	- + +	- + +	- + +	0 4 16

4.4 RESULTS

Following reindeer infection, stress and aggressiveness were noted for reindeer 1 and 3 for about 2 weeks. Reindeer 2 kept himself isolated from the others more than usual, and frequently exhibited panting, pacing, and dragging his feet. Behavior returned to normal for all the reindeer in the following weeks. Postmortem examination did not show any abnormalities, excepted for the unexposed control that died of enteritis caused by *Clostridium perfringens*. His tissues were kept as a negative control, and no evidence of *T. gondii* was detected after serological and molecular testing. Reindeer 3 had to be euthanized early due to welfare reasons (at 16 vs 20 weeks pi). All reindeer were seronegative before inoculation, and all experimentally infected reindeer demonstrated seropositivity 4 weeks after infection and remained seropositive at time of euthanasia (see Table 6). One *T. gondii* tissue cyst was visualized on a tissue squash of the brain of reindeer 2 (see Figure 5). Histopathology and immunohistochemistry were performed on heart, liver, spleen, lung, kidney, brain, tongue, diaphragm, triceps, external oblique, gluteus medius, and longissimus of the infected reindeer. *Toxoplasma gondii* cysts were visualized by immunohistochemistry on tongue, diaphragm, external oblique, and triceps of reindeer 3, and on external oblique and gluteus medius in reindeer 1 (see Figure 3 and 4). Pleuritis and alveolitis were visualised in reindeer 1 after histopathology with the following changes: focal to coalescing chronic fibrinous eosinophilic histiocytic pleuritis, with mild focal eosinophilic alveolitis (see Figure 6). No changes were detected in the control reindeer (examined at 7 days pre-infection) after histopathology on heart, liver, spleen, lung, kidney, rumen, and brain.

Following conventional PCR on traditional DNA extraction, *T. gondii* was detected in brain, diaphragm, lung, gluteus biceps, longissimus, and triceps in reindeer 1, in longissimus, external oblique, and triceps in reindeer 2, and in brain, diaphragm, lung, tongue, gluteus biceps, gluteus medius, external oblique, and triceps in reindeer 3. Following magnetic capture, DNA was detected in all tissue samples tested: longissimus, gluteus medius, gluteus biceps, external oblique, triceps, diaphragm, tongue, brain, heart, and lung (see Table 7). Both methods were compared when the same organs and muscles were

tested, with a total of 26 samples. The overall tissue prevalence detected by a combination of traditional DNA extraction and conventional PCR was 61.5% (16/26), and 100 % (26/26) for magnetic capture.

Table 7. Detection of *T. gondii* DNA in different organs comparing two DNA extraction methods

		Traditional DNA extraction (25mg)	Magnetic capture (100g)
Reindeer control	Heart	-	Not tested
	Tongue	-	Not tested
	Gluteus biceps	-	-
Reindeer 1 (1000 oocysts)	Brain	√	√
	Heart	-	√
	Diaphragm	√	√
	Lung	√	√
	Tongue	-	Not tested
	Gluteus biceps	√	√
	Gluteus medius	-	√
	Longissimus	√	√
	External oblique	-	√
	Triceps	√	√
Reindeer 2 (5000 oocysts)	Brain	-	√
	Heart	-	√
	Diaphragm	-	√
	Lung	-	√
	Tongue	-	Not tested
	Gluteus biceps	-	Not tested
	Gluteus medius	-	√
	Longissimus	√	√
	External oblique	√	√
	Triceps	√	√
Reindeer 3 (10000 oocysts)	Brain	√	√
	Heart	-	√
	Diaphragm	√	√
	Lung	√	√
	Tongue	√	Not tested
	Gluteus biceps	√	√
	Gluteus medius	√	√
	Longissimus	-	√
	External oblique	√	√
	Triceps	√	√

√: positive - : negative

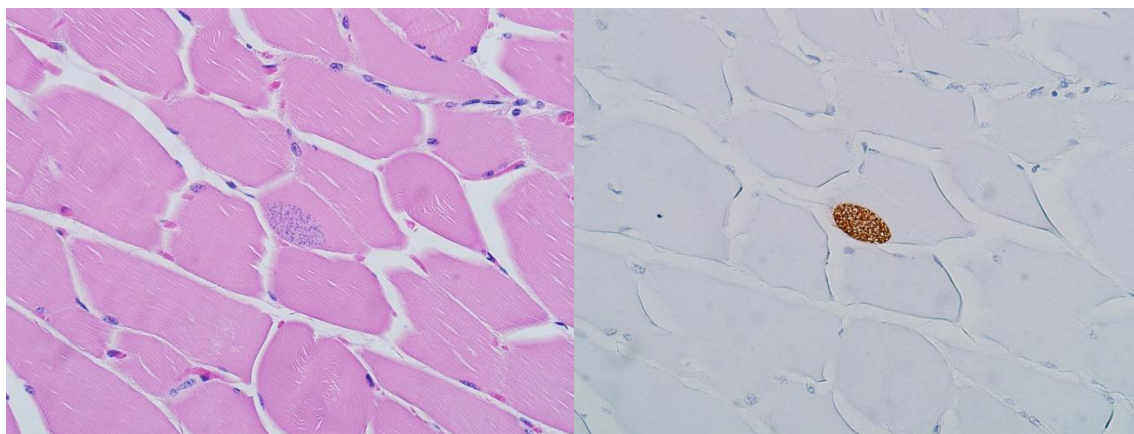


Figure 3. Histological section of reindeer (no. 1) muscle containing a *T. gondii* cyst visualised at 40X after HE (on the left) and immunohistochemical stain (on the right).

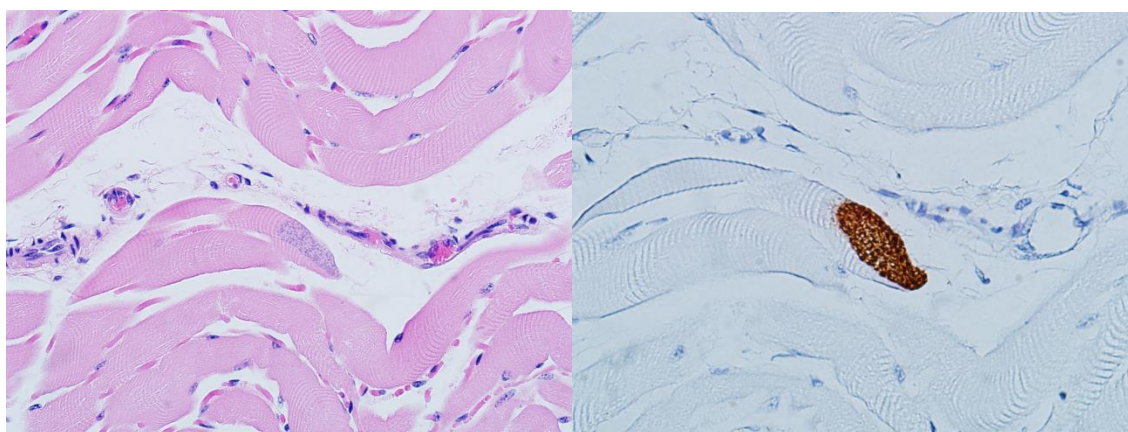


Figure 4. Histological section of reindeer (no. 3) diaphragm containing a *T. gondii* cyst visualised at 40X after HE (on the left) and at 60X after immunohistochemical stain (on the right).

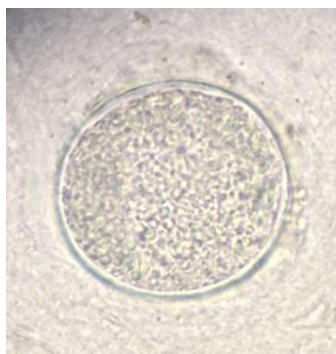


Figure 5. Picture of *T. gondii* tissue cyst of reindeer (no. 2) brain visualized on compound microscopy (60X).

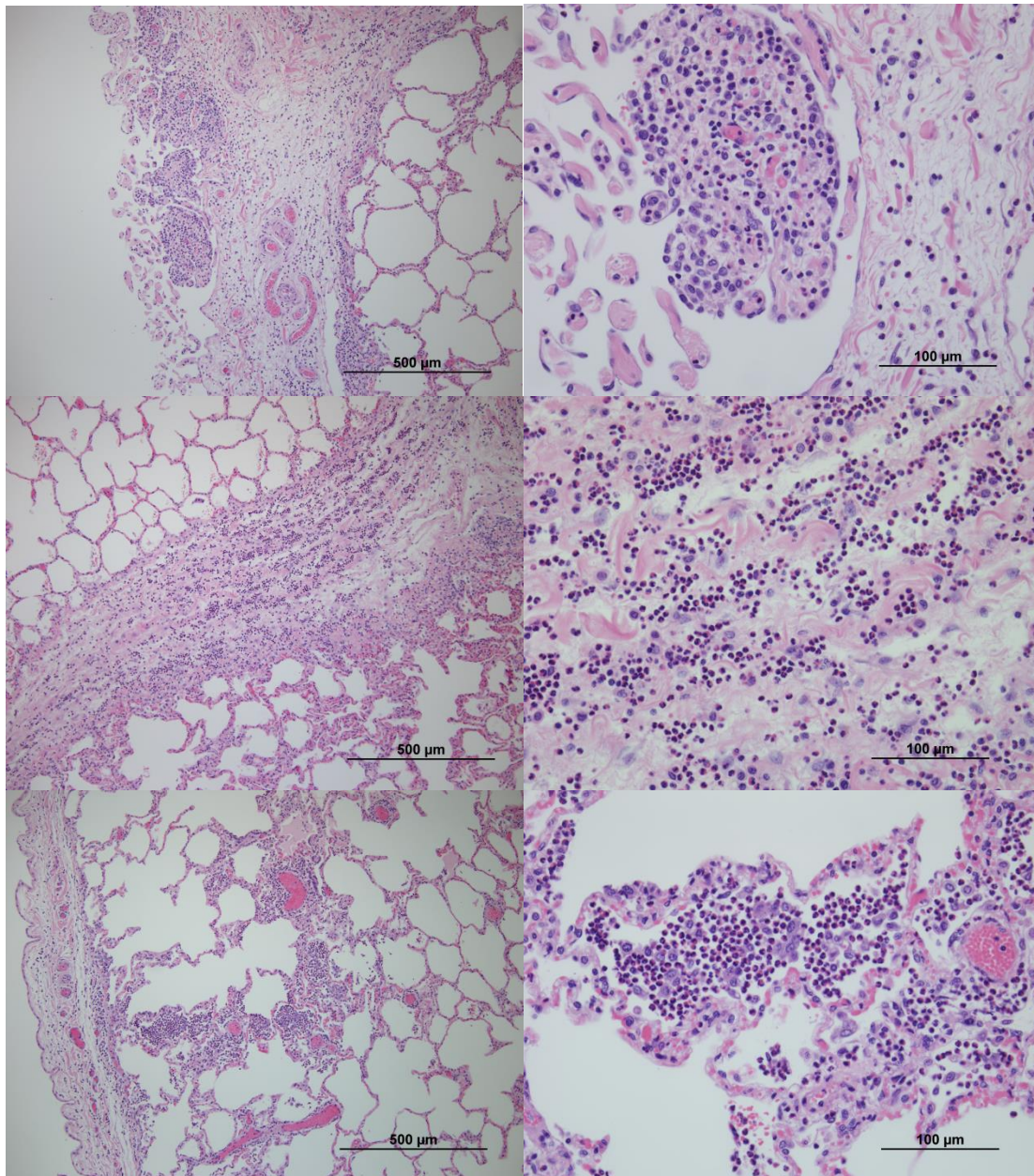


Figure 6. Histological section after HE of reindeer (no. 2) pleura at 10X (top left) and 40X (top right), of septa at 10X (middle left) and 40X (middle right), and of alveoli at 10X (bottom left) and 40X (bottom right). Focal to coalescing areas of chronic changes are noted, with fibrinous material, eosinophils and histiocytes.

4.5 DISCUSSION

This study reported the first experimental infection in reindeer inoculated orally with oocysts of *T. gondii* Type III via nasogastric tube, and successfully established subclinical infections. Minor behavioral changes were observed for a few days following infection, but this may reflect changes in housing and stress due to handling. Our observations demonstrated that reindeer are good intermediate hosts for *T. gondii* and susceptible to sporulated oocysts, as described previously in the literature (Oksanen et al., 1996). The only other experimental infection in reindeer resulted in a marked acute rumenitis that may have been associated with intraruminal inoculation of the ME-49 strain of *T. gondii*. Oksanen et al. (1996) inoculated the animals via intraruminal injection through the abdominal wall. Even though the gastrointestinal route was used, our study might reflect more the natural route of infection by “feeding” oocysts to the animals. Furthermore, oocysts of the ME-49 strain are considered virulent as demonstrated previously (Dubey et al., 1995a). With the strain used in our work (VEG III strain), subclinical infections were previously successfully achieved (Gajadhar et al., 2004; Dubey et al., 2012), although demonstration of lethal doses ranging from 10 to 1000 oocysts were seen in BALB/c mice (Gajadhar et al., 2004; Dubey et al., 2012). It is thus possible that the difference in pathology observed in both reindeer experimental studies and the lack of severe disease in ours have been due to the low dose of oocysts given, host susceptibility to VEG strain of *T. gondii* and differences in strain virulence, as well as the method of inoculation. Even though our reindeer seemed to tolerate infection well, behavioral changes observed would not have been detected in the wild and can have important implications in wild populations of caribou. In intermediate hosts, it has been shown that *T. gondii*-infected rodents exhibit an increase in activity and a decrease in predator vigilance behavioural traits, therefore increasing their risk of predation (Hay et al., 1983; Hay et al., 1984; Berdoy et al., 1995; Lamberton et al., 2008). *Toxoplasma gondii* preference for the central nervous system of its intermediate host places it in a privileged position to manipulate host behavior (Webster, 2007; Webster et al., 2013). Increase risk of predation in sea otters by sharks has also been described following infection with the parasite (Miller et al., 2004).

Inflammation in the lungs of the second dose infected reindeer was seen following histopathology. This could explain the fact that he was panting and seemed more depressed than the others. Lung inflammation following *T. gondii* infection has been described in the literature (Hirth and Nielsen, 1969; Pomeroy and Filice, 1992; Filice et al., 1999; Leal et al., 2007; Dubey, 2010b; Shen et al., 2015). No changes were associated with tissue cysts seen with immunohistochemistry. Regarding IHC, we noted more cysts in the reindeer given the highest dose of oocysts, with four positive stained tissues. It has been shown that more tissues cysts are present in tissues of animals that were infected with higher doses of oocysts of the VEG strain of *T. gondii* (Dubey, 1996b; Dubey et al., 1996). Cysts were observed by IHC on two different muscles in reindeer 1 and one cyst was observed in the brain of reindeer 2. Differences in cyst amount could be explained by the fact that we only used a small amount of tissue fixed on one slide for each organ when performing IHC. As few as one cyst can be present in an entire organ (Hill and Dubey, 2002). The same explanation applies to tissues tested with molecular techniques. Finally, as only three reindeer were tested, it is difficult to draw conclusions about the distribution of parasites.

As expected, we found a higher prevalence of *T. gondii* in tissue using DNA extracted via magnetic capture followed by conventional PCR, than with a commercial kit (100% vs 61.5%). Using the MC technique, DNA of *T. gondii* was detected in all organs tested, showing that conventional PCR targeting the 529bp repeat element can thus be a sensitive method used in combination with MC technique. Because 100g can be used for magnetic capture isolation, a higher probability of *T. gondii* detection was expected using this technique instead of traditional DNA extraction from 25 mg of tissue. We suggest the use of the MC technique when large amounts of tissue are available, and it can be a very useful screening tool in exposure assessment in wildlife and other animals intended for human consumption. In a previous study, this technique has shown an equal sensitivity compared with the mouse bioassay, which is often

considered to be the gold standard for detection of *T. gondii* in meat samples (Garcia et al., 2006; Opsteegh et al., 2010).

DNA of *T. gondii* was detected in all organs tested, suggesting that the doses we used were high relative to natural infections where only a few organs and tissues might harbour tissue cysts. Oksanen et al. (1996) used 5,000 and 50,000 oocysts to infect reindeer and draw the same conclusion as they were not expecting clinical toxoplasmosis with this amount of oocysts. Reindeer in their environment might only be exposed to low numbers of *T. gondii* oocysts since natural clinical toxoplasmosis are rarely seen. Therefore, the presence of *T. gondii* in multiple tissues following experimental infection might not reflect the distribution of the parasite in naturally infected caribou in the wild. Experimentally infected animals have less variation in infectious dose, time after infection, and host and parasite genetics than with naturally infected animals (Opsteegh et al., 2010). In our study, we used a moderately virulent terrestrial Type III VEG strain given orally in order to establish subclinical infection and avoid acute toxoplasmosis. Caribou migrating south might be exposed to different strains, less or more virulent, and experience different clinical signs. There is no well documented report of natural clinical toxoplasmosis in *Rangifer*, which could reflect relatively low levels of environmental contamination with *T. gondii* oocysts throughout their migration range. However, a high prevalence of antibodies (37%) has been detected in mainland caribou (Kutz et al., 2001), suggesting relatively high levels of exposure. Since primary infected cats can shed 1 to 50 million potentially infectious oocysts in the environment, it is hard to quantify environmental contamination in a specific area. Levels of contamination can vary greatly along the urban-rural-wild gradient (Gilot-Fromont et al., 2012). One study from the Morro Bay area in California estimated the annual burden in the environment between 94 and 4671 oocysts/m² (Dabritz et al., 2007). Another study in France estimated rate of soil contamination by *T. gondii* oocysts between 31 and 3600 oocysts/m² per year.

In this experimental infection study, we detected the parasites in multiple muscles and organs commonly consumed by northern communities. Caribou is consumed in every Inuit community across Canada, with preferences depending on the region. As a nutrient dense food, practically all parts of caribou are traditionally eaten which consist in meat, milk, organs, blood, bone marrow, and oil (Wein and Freeman, 1992; Wein et al., 1996; Meis Mason et al., 2007; Department of Health, Revised in 2013). Inuit in Canada's north traditionally relied on "country food" (hunting caribou, seal, fish, wild berries etc). However, modernization and the high cost of hunting are changing Inuit eating habits, with store-bought foods being increasingly popular in Inuit communities (Rosol et al., 2011; Sheikh et al., 2011; Huet et al., 2012). Nevertheless, traditional foods still remain frequently consumed, highly preferred, and an integral part of the social and cultural way of Inuit life (Wein and Freeman, 1992; Wein et al., 1996; Kuhnlein et al., 2004; Meis Mason et al., 2007). Meat and organs are often eaten raw, dried, or smoked (McDonald et al., 1990; Messier et al., 2009). These findings in our reindeer experiment can help communities to implement effective strategies to prevent human infection, including safer methods of food preparation for high risk people, such as pregnant women. Future work could include characterizing tissue distribution of *T. gondii* in naturally infected caribou as well as determining the effects of traditional methods of food preparation on viability of tissue cysts, although the collection of such samples might be logistically challenging. With caribou populations declining, stressed by climate and anthropogenic landscape changes, further studies are needed to fully understand the epidemiology and clinical significance of toxoplasmosis in free-ranging caribou. Climatic and meteorological conditions as well as level of anthropization are significant factors explaining the spatio-temporal variations of *T. gondii* in wild populations (Almeria et al., 2004; Gamarra et al., 2008; Afonso et al., 2010; Jokelainen et al., 2010; Richomme et al., 2010; Jokelainen et al., 2011; Malmsten et al., 2011).

In summary, we successfully demonstrated subclinical infection of *T. gondii* in reindeer experimentally infected via naso-gastric tube with three different doses of oocysts of the VEG strain

(Type III). Tissue cysts were detected in all organs and muscles tested via magnetic capture, suggesting the use of this highly sensitive technique in wildlife when large quantities of tissue are available. This can be a valuable alternative to mouse bioassay, which is often time consuming and ethically challenging, although it does have the added bonus of demonstrating viability. We found a wide distribution of the parasites in multiple organs and muscles, even in the reindeer infected with the lowest dose. All of these organs and muscles are commonly consumed by indigenous communities (Condon et al., 1995; Meis Mason et al., 2007). However, the dose of oocysts given orally might not reflect the environmental contamination in the wild which probably leads to lower infection rates. Nevertheless, 1000 oocysts succeeded to infect all tissue tested, it thus raises concern and suggests that no “safe” tissue is present in an infected animal. These findings emphasize the importance of performing further studies on the distribution, concentration, and viability of *T. gondii* in tissues of naturally infected caribou in order to assess the risk of human and animal infection associated with handling or consumption of caribou. Even though we did not see any major detrimental changes in health or behaviour of captive reindeer following infection, we would not have detected subtle behavioural changes associated with toxoplasmosis that could predispose wild caribou to predation or act synergistically with stress from other sources, such as climate change and industrial development and other diseases and parasites. Finally, toxoplasmosis may have its greatest impact on fetuses of acutely infected dams, causing abortion and neonatal illness and thereby decreasing reproductive success of already declining caribou populations.

CHAPTER 5: General Discussion and Conclusion

The main goal of this research was to determine major routes of transmission of *Toxoplasma gondii* in a terrestrial Arctic ecosystem undergoing rapid climatic and anthropogenic changes. These changes have the capacity to enhance transmission via increased precipitation, and changes in host ecology, thus increasing risk of contracting the parasite for wildlife and people in the North (Meerburg and Kijlstra, 2009). As the parasite causes abortion and congenital disease in multiple species, it is a concern for wildlife and public health.

In Chapter 2, we looked into seroprevalence and congenital way of transmission in Arctic foxes in order to better understand how Arctic wildlife is exposed, if it causes problems or not, and how the parasite maintains itself in High Arctic. We demonstrated for the first time the possibility of vertical transmission in Arctic foxes in the wild and that it can happen in more than one litter from the same mother, suggesting another way of transmission beside food-borne routes and potential for effects on reproductive success in wildlife species of conservation concern. Arctic-nesting geese in the Karrak Lake ecosystem are also exposed to the parasite and since they are an important part of the diet of these foxes, I looked into multiple ways of detection (as part of Chapter 3) by comparing serological and molecular methods on goose samples. By using different types of samples and multiple detection methods, we take in account the lack of a gold standard in wildlife studies, and will help further work in determining the significance of Arctic-nesting geese as a source of transmission in northern ecosystems. I also used a serological test on tissue fluid in the field for the first time (modified agglutination test) and proved that it can be used successfully in the field and in northern communities to screen for potential contaminated meat intended for consumption in the North.

We demonstrated exposure to *T. gondii* in foxes and geese in Chapter 2 and 3. Foxes could be exposed by scavenging on geese, as well as other wildlife, like caribou. As for the geese, caribou are migratory animals and their meat is an important food source in the Arctic. In Chapter 4, I looked into what effects the parasite could have on the health of experimentally infected reindeer, conspecific with

caribou. I found a wide distribution of *T. gondii* in all organs and muscles tested, also commonly consumed by northern people. These findings emphasize the importance of performing further work in naturally infected caribou regarding the risk associated with handling and consumption of these animals. Subtle behavioral changes and respiratory problems were noted immediately post infection, meaning that *T. gondii* can potentially affect the health of wild caribou populations. Even though the changes observed were not severe, *T. gondii* could increase stress of a population already in decline. Even sub-clinical changes in behavior or fitness could be critical for free ranging caribou that are subject to predation and other stressors.

Foxes, geese and caribou are an inherent part of the Arctic, being a cultural and socioeconomic cornerstone for northern people. As key species in the North, and with climate change modifying polar regions, it is critical to keep investigating *T. gondii* and determining what effects it may have in wildlife population health as well as for Arctic people. More information on food-borne transmissions of *T. gondii* is needed to put into practice culturally appropriate and effective prevention measures.

5.1 Transmission and diversity of *T. gondii* in a terrestrial Arctic ecosystem

Toxoplasma gondii is a protozoan parasite with worldwide distribution and is ubiquitous among vertebrates (Dubey, 2009d). Domestic and free-ranging felids are the only recognized definitive hosts for *T. gondii* in which the parasite can achieve sexual reproduction, excreting oocysts in feces. If an intermediate host ingests sporulated oocysts from contaminated food, water, or the environment, they will develop cysts in their tissues. Asexual reproduction may then occur in both intermediate and definitive hosts via carnivorous or vertical transmission (transplacentally) (Robert-Gangneux and Darde, 2012). *Toxoplasma gondii* has been found in terrestrial Arctic ecosystems despite the fact that wild and domestic cats are present at lower density than in southern latitudes (Elmore et al., 2012). Previous studies have successfully demonstrated *T. gondii* seroprevalence in Arctic foxes, as well as in Arctic nesting geese at

Karrak Lake, Nunavut. Geese might be a potential source of introduction into this ecosystem, possibly infecting foxes through carnivorism as well.

5.1.1 The role of vertical transmission of *T. gondii* in Arctic foxes (i.e., female foxes to the pups).

For this research, the groundwork already established in Karrak Lake ecosystem allowed us to look into another way of transmission of *T. gondii*, namely congenital transmission. We collected blood samples from Arctic foxes and their respective litters and found antibodies in both, reporting thus what is likely to be the first natural vertical infection in neonate Arctic foxes. Vertical transmission of *T. gondii* has been reported in domestic animals and captive wildlife before (Tenter et al., 2000; Duncanson et al., 2001; Marshall et al., 2004; Hide et al., 2009). One study reported transplacental toxoplasmosis and congenital cerebral malformation in a stranded wild neonatal sea otter found without its mother (Miller et al., 2008a), but no study has ever demonstrated vertical transmission in free ranging wildlife by looking at both the mother and unweaned offspring. Furthermore, we found that both litters with positive pups were from mothers who had been seropositive the year previously, which is contrary to the assumption that congenital infection can only happen in one litter over a lifetime. Transmission in multiple pregnancies has been reported in deer mice and sheep (Morley et al., 2008; Rejmanek et al., 2010); however, it is largely assumed that trans-placental infection rarely occurs when *T. gondii* is acquired for the first time before pregnancy (Remington et al., 2004; Rahman et al., 2015).

Occurring in early gestation, transplacental transmission may lead to multiple problems including abortion, neonatal death, or foetal abnormalities (Tenter et al., 2000). Infection and deaths of Arctic foxes in the wild caused by this protozoan has been reported before (Prestrud et al., 2007; Prestrud et al., 2008b). Infected vixens and death of neonates have also been demonstrated in multiple farm foxes, as well as high mortality rate in newborns of experimentally infected blue foxes (Bjerkas, 1990; Smielewska-Los et al., 2000). Regarding our study, further work is needed to determine if maternal antibodies could have been present in pups, if the whole litter is usually infected, if transmission routinely

occurs in multiple litters from the same animal, and what effects the parasite has on pup survival inside the mother and in early life.

5.1.2 Long term *T. gondii* serostudy to explore interannual dynamics in Arctic foxes.

Besides vertical transmission, recaptures of adult foxes give valuable information regarding when animals become seropositive in their life and antibody persistence in naturally infected animals. By looking at long term data, we saw a shift from positive to negative in serostatus of one fox, which suggest that antibodies may fade over time. Antibodies against *Toxoplasma* are usually thought to persist for a lifetime in its host due to constant antigenic stimulation from persistent tissue cysts (Remington and Krahenbuhl, 1982; Opsteegh et al., 2011). No study has looked into long term exposure in wild animals to see if antibodies last that long. Since foxes from the Karrak Lake region have been studied for many years, it would be valuable to keep looking into seroprevalence throughout time to see if antibodies change, if it is common or not, and when foxes get exposed. We also found that mature animals (≥ 2 yr) are more likely to be exposed to *T. gondii* and seroconvert than young foxes, as has been described in previous studies on foxes, other animal species, and humans as well (Lin, 1998; Mitchell et al., 1999; Dubey and Jones, 2008; Elmore et al., 2016b). It suggests that foodborne transmission is probably important for maintenance of the parasite. Finally, Arctic fox may serve as indicators for potential human exposure and transmission of *T. gondii* in the North.

5.1.3 Genetic diversity of *T. gondii* in migratory geese and foxes at Karrak Lake.

Toxoplasma gondii transmits in a prey-predator system that alternates between definitive (sexual reproduction happening only in felids) and intermediate (asexual replication) hosts (Robert-Gangneux and Darde, 2012). The fact that *T. gondii* has a highly clonal nature provides evidence that other ways of transmission often bypass sexual reproduction in the definitive host, whether it is by carnivorous or vertical transmission (Sibley and Boothroyd, 1992; Hide et al., 2009). To investigate *T. gondii* genotypes

in Arctic foxes would give us information about the degree of clonality of *T. gondii* strains in infected animals and elucidating the population structure and transmission mode of this parasite.

In North America and Europe, most strains of *T. gondii* isolated are found to be clonal and grouped into 3 (Types I, II, III) genotypes (Dubey et al., 2010). In our work, no sequences were found in any goose samples tested, thus no genotyping could have been achieved. It might reflect a low infection status in these Arctic-nesting geese as well as the limitations of the conventional PCR used. However, *T. gondii* DNA in this bird population has successfully been sequenced and genotyped previously, finding clonal genotype III in brain and heart (Elmore et al., 2016a). We would have expected clonal genotype Type III since it is commonly associated with agriculture land where migrating geese are known to feed on their way to their breeding grounds in the Arctic (Leafloor et al., 2012). A fourth clonal type (Type 12) has been found in wildlife in North America (Dubey et al., 2011b). This genotype could be present in Arctic-nesting geese, although Type III is consistent with the geese feeding ecology (Elmore et al., 2016a). In addition to Type 12, Type II and atypical genotypes from Alaska have also been described (Dubey et al., 2010; Dubey et al., 2011b). Finally, a Type X has been described in marine and coastal environments and is associated with clinical toxoplasmosis affecting sea otter populations in California (Miller et al., 2008b). Beside this, more information is needed concerning genotypes in northern ecosystems. In Arctic foxes from Svalbard, Norway, where no cats are present, Type II has been predominantly isolated (Prestrud et al., 2008a; Prestrud et al., 2008b). In Karrak Lake fox population, Type III would be expected since they are known to scavenge on migratory geese.

Although this objective (to characterize genetic diversity of *T. gondii* at Karrak Lake) was not achieved in this work, future work could involve getting fox carcasses from trappers. Performing molecular work on tissues would give helpful information regarding infection vs exposure.

5.2 Comparison of serological and molecular techniques: the use of multiple tests, new perspective for remote studies, and magnetic capture DNA extraction maximizes detection probability

5.2.1 To compare serological techniques (MAT vs IFAT) in the lab for Arctic fox blood samples.

Concerning assays performed on fox sera, the use of two different tests increased our confidence in detection, with both assays (i.e., MAT and IFAT) giving us similar results with an overall apparent seroprevalence in adults of 54% and of 28% in pups. Previous work on Karrak Lake adult foxes demonstrated a detection probability using IFAT at 72%, MAT at 61% and ELISA at 46% (Elmore et al., 2016b), indicating IFAT as the test of choice. MAT and IFAT have both been commonly used in wildlife, and have the advantage of being flexible for multiple species (Dabritz et al., 2008; Miller et al., 2008b). However, IFAT often requires a taxon-specific second antibody which has not always been available commercially (Elmore et al., 2016b). Bias can also occur regarding results based on visual inspection for both tests.

5.2.2 To compare molecular and serological methods in wild Arctic-nesting geese in Karrak Lake ecosystem.

The lack of gold standard in *T. gondii* serology in wildlife as well as the possibility of cross reactivity with other coccidians makes it difficult to validate any results (Elmore et al., 2014; Shapiro et al., 2015). Depending on the test used, the sample types, and storage conditions, results may often vary. The use of multiple tests can increase chances to find true positives. Our estimates for Ross's and Lesser Snow Geese (20% and 12.5% respectively for thoracic fluids in the field; 14.8% and 9.5% respectively for frozen/thawed thoracic fluids in the lab; 3.1% and 3.4% respectively for frozen/thawed heart fluids; 0% and 0% respectively on blood on filter paper strips), are lower than previous estimates in this ecosystem (32.4% and 28.3% respectively on serum; 39% and 36% respectively on blood on filter paper strips)

(Elmore et al., 2014; Elmore et al., 2015); however, *T. gondii* is present in Arctic nesting geese at Karrak Lake, which may be exposed along their migratory route and carry it to their breeding grounds (Elmore et al., 2014; Elmore et al., 2015; Elmore et al., 2016a). This hypothesis has been examined in Svalbard, Norway, where Arctic foxes have shown a seroprevalence of 43% to the parasite (Prestrud et al., 2007).

Our best estimate was found on fresh thoracic fluids tested on the field using a new modified agglutination test (MAT). The results suggest that samples from dead wildlife are often suboptimal, and that common methods of collection (i.e., filter paper), and freezing samples may underestimate seroprevalence of *T. gondii*. False negative results could have ensued from the high dilution performed on our samples. The use of filter paper has given good results in previous studies (Curry et al., 2011; Curry et al., 2014; Elmore et al., 2014), and was successfully used in a remote area (Aston et al., 2014). However, Curry et al. (2014) found that filter papers stored in dry conditions or at room temperature were less sensitive than serum and that testing at 6 months provided better results than over 12 months. Nevertheless, the sensitivity was still high when subjected to these conditions. The use of sera instead of body fluids which are often heterogeneous would also be preferable for testing since body fluids often contain particulate matter that might give rise to false positives. False positives could have occurred regarding the risk of crossreactivity with unknown non-target antibodies as well as results based on visual inspection. It could explain why one sample was positive when tested in laboratory but not in the field. Finally, serology remains our only indication of exposure to *T. gondii* for this study, underlining the many challenges of detection and recovery for molecular characterization of tissue dwelling coccidia.

5.2.3 To compare a serological method (MAT) in the field versus laboratory for wild Arctic-nesting geese using body fluids.

I also used a serological test in the field for the first time (modified agglutination test) and proved that it can be used successfully in the field and in northern communities to help screen for potential contaminated meat consumed in the North. However, I recommend the use of serum or whole blood

whenever possible instead of body fluids since heterogeneous matter is often present when collected in the thoracic cavity and not always visible with the naked eye. The quality of such samples can affect the results by giving false positives. Otherwise, the use of this technique in remote areas can be beneficial for a public health and food safety point of view. Although creating a prevention program for *T. gondii* in northern communities might be challenging from a communication, training, and funding aspect, it has been successfully implemented in Nunavik for another food-borne parasite, *Trichinella nativa*, transmitted via consumption of walrus meat (Larrat et al., 2012).

5.2.4 To compare traditional vs magnetic capture PCR for detection of DNA of *T. gondii* in tissues of wild geese and experimentally infected reindeer.

The molecular diagnostics on goose tissues were inconclusive. Low infection rates in this goose population could explain the results, as well as the limitation of conventional PCR and the small sample size. After performing a trial with magnetic capture DNA extraction on goose brain and heart pooled together, and spiking test samples with *T. gondii* tachyzoites, we confirmed a minimal detection of 50000 tachyzoites per 100 g of tissue following conventional PCR targeting the 529bp repeating element (Homan et al., 2000). Magnetic capture followed by quantitative real-time PCR detected a minimum of 230 tachyzoites per 100 g of meat (Opsteegh et al., 2010). We thus suggest the use of MC capture followed by qPCR when possible to increase detection. Unlike traditional kits used for DNA extraction, magnetic capture has the benefit of isolating pure parasite DNA, which minimizes production of nonspecific PCR product or inhibition due to excessive amount of host DNA (Jurankova et al., 2014b). However, magnetic capture is more time consuming and costly compared to commercial kits for DNA extraction. Conventional PCR targeting the 529bp element was used after both extraction methods (Homan et al., 2000). Magnetic capture allowed the use of 100g of tissue instead of 25mg with a kit-based method of extraction. The MC technique was first used to isolate *T. gondii* DNA from experimentally infected pigs and naturally infected sheep (Opsteegh et al., 2010). It was also applied to test for goat, pig,

and turkey infections (Jurankova et al., 2013; Jurankova et al., 2014a; Koethe et al., 2015). One study in wild house mice was also performed (Jurankova et al., 2014b).

Finally, we were able to detect the parasite in all 26 organs and muscles of reindeer tested with the MC technique (100%), compared to 61.5% with commercial kit of DNA extraction. Magnetic capture has not been widely described in wildlife yet; we thus recommend the use of this technique in wild animals to increase detection probabilities. It is also a good alternative to bioassay in mice which is considered the gold standard in analysis of meat samples. Bioassays are often laborious, time consuming, and ethically challenging (Opsteegh et al., 2010); however, they have the benefit of demonstrating viable organisms instead of DNA only.

5.3 Experimentally infected reindeer demonstrated a wide distribution of *T. gondii* in various tissues with minor clinical effects

Previous studies established a strong correlation between eating raw caribou meat and toxoplasmosis in people in Nunavik (McDonald et al., 1990; Messier et al., 2009). Therefore, we decided to experimentally imitate natural *T. gondii* infection in reindeer via the oral route, and to look into which tissues the parasite would be located predominantly. Only one study described experimental infection in reindeer, resulting in fatal enteritis in the subjects (Oksanen et al., 1996), more likely due to the large infective dose of oocysts given as well as the intraruminal method of inoculation.

5.3.1 To determine clinical effects and pathology of *T. gondii* in experimentally infected reindeer.

This work was the first successful establishment of subclinical infection of *T. gondii* in captive reindeer. No major detrimental effects were seen in experimentally infected reindeer besides increase of aggression and stress for a few days following infection, and some changes in the lungs in one reindeer. Respiratory problems have been described in the literature following *T. gondii* infection in naturally and

experimentally animals (Hirth and Nielsen, 1969; Pomeroy and Filice, 1992; Filice et al., 1999; Leal et al., 2007; Dubey, 2010b; Shen et al., 2015). After experimentally infecting grey seals with the VEG strain, Gajadhar et al. (2004) also noted mild behavioral changes in animals without severe health problems. Depending on the infectious dose, the strain used, and the host and parasite genetics, outcomes may vary greatly between studies (Opsteegh et al., 2010).

Environmental stressors (e.g., climate change, anthropogenic factors, diseases) can favor transmission of pathogens in animals. Following infection, risk of predation and susceptibility to other disease and parasites might increase. Previous studies in mice and sea otters demonstrated that after becoming infected, animals were actually becoming less risk adverse to predation (Miller et al., 2004; Webster, 2007; Webster et al., 2013). It has also been suggested that maternal body condition of female caribou influences susceptibility to climate-related events and, subsequently, risk from predation for her and her young (Bastille-Rousseau et al., 2016). Calving season starts usually towards the end of May and caribou will migrate south in Fall (August-October) (Gustine et al., 2006), often below the tree line where females can possibly be exposed to environmental contamination by *T. gondii* oocysts. With rut occurring in between October and late November, females can thus become infected at the beginning of pregnancy. In non-immune animals, this could mean an increase in neonatal illness and abortion, decreasing reproductive success. With caribou populations already in decline, *T. gondii* could impact calving success as well as their health, as well as for communities depending on this valuable food source. Finally, the lack of major clinical signs and pathology means that reindeer/caribou are good hosts for *Toxoplasma* and may have public health significance.

5.3.2 To determine tissue predilection in experimentally infected reindeer.

In our research, *T. gondii* was successfully detected in commonly consumed muscles of reindeer orally infected with three different doses of oocysts (1000, 5000, and 10000). Although we were limited in our sample size and the doses given might be higher than in the environment (estimation in between 30

to 4700 oocysts/m² have been described before in rural and urban region of California and France (Dabritz et al., 2007; Gilot-Fromont et al., 2012)), this gives us important insight into food-borne transmission of *T. gondii* in northern communities. However, further studies looking into naturally infected animals would be more representative of the reality since doses and strains may vary in the environment. Taking into account conservation concerns, unknown exposure dose, and logistical challenges in acquiring samples, to carry out such research in wild caribou would be very challenging. In addition, looking into traditional ways of preparing caribou meat (i.e., dried or smoked) and the viability of the parasite after preparation would also give valuable information regarding risk of food borne transmission. Such study has been performed on seal meat previously (Forbes et al., 2009), but not on caribou.

With magnetic capture, we were able to detect the parasite in all organs and muscles examined, regardless of the infective dose. In the literature, heart and brain are often the two main predilection sites of *T. gondii* (Esteban-Redondo and Innes, 1998; Robert-Gangneux and Darde, 2012; Elmore et al., 2016a); we thus expected to find the parasite predominantly in these two organs. For all Indigenous peoples, traditional food is at the heart of culture and health. Even though it is recognized as an essential part of life, the all-Arctic Canadian proportion of dietary energy from traditional food consumed in one day was estimated downwards from 23.4% in 1999 to 16.1% in 2008, with caribou being the most consumed meat source in 2008 (32%) followed by Arctic char (13%) (Kuhnlein et al., 2004; Sheikh et al., 2011). These surveys were conducted in 18 Inuit communities in 5 regions (Inuvialuit Settlement Region; Nunavut's Kitikmeot, Kivalliq and Qikiqtaaluk [Baffin] Regions; and Labrador). The decrease in traditional food consumption over the years can be explained by changes in lifestyle, food insecurity, poverty, the effects of climate change and the bans on fur trading. Nevertheless, country food is considered to be part of the environmental entity in which families live and remains crucial for providing many essential nutrients in Inuit communities (Huet et al., 2012). Caribou are universally present in practically all Canadian Inuit ecosystems (Sheikh et al., 2011). Consumption of muscles (legs, shoulder, ribs, rump, front end, breastbone, backbone), fat, tongue, head, stomach content, heart, kidney, and liver

has been demonstrated (Condon et al., 1995; Wein et al., 1996). In fact, all parts of caribou are generally consumed and used (Meis Mason et al., 2007). Our detection in all reindeer tissues tested underlines the importance of performing further studies on the distribution, concentration, and viability of *T. gondii* in naturally infected caribou. This is crucial to assess the risk of human and animal infection associated with handling or consumption of caribou.

5.4 Conclusion

With global climate change and anthropogenic factors affecting the Arctic at higher rates than anywhere else on the planet (Rinke and Dethloff, 2008), especially in the western Canadian Arctic where my work was performed (Hoberg et al., 2008), the prevalence of *T. gondii* could be increasing in northern latitudes (Meerburg and Kijlstra, 2009). According to Meerburg et al. (2009), further urbanization in the North may increase interaction between domestic or stray cats and wild animals affecting the presence of the parasite in the environment. The augmentation of precipitation may play a role as well by changing ecological patterns of host species, and also via increase of freshwater and marine runoff that may contain infective *T. gondii* oocysts. Range expansion of insects that can act as transport hosts may also contribute to increase transmission (Chinchilla et al., 1994; Meerburg and Kijlstra, 2009). Understanding how the parasite thrives in this specific ecosystem is crucial in order to establish effective prevention measures for northern people, and better monitoring of key wildlife species, such as **Arctic fox, geese, and caribou**. Caribou population are declining throughout their circumpolar range, and as an inextricable part of northern culture and economy, the decline of this species will remove a significant source of food and income for northern communities (Vors and Boyce, 2009).

In this work, I discovered the possibility of vertical transmission in multiple litters of wild **Arctic foxes**, which could help us to understand *T. gondii* success in species with practically no contact with the definitive hosts, the cats. The parasite might be introduced by migratory geese infected on their wintering grounds and during their migration routes (Elmore et al., 2016b). Adult Arctic foxes could in turn become infected by scavenging on infected geese and other wildlife, such as caribou. Exposure through oocysts in water remains a possibility although Karrak Lake fox populations are more characterized as inland, versus coastal foxes that depend on marine resources for survival (Samelius, 2004; Samelius et al., 2007). Knowing that the parasite may be transmitted congenitally in addition to food-borne routes gives more insight regarding how the parasite thrives in the Karrak Lake ecosystem.

I demonstrated evidence of exposure to *T. gondii* in **Arctic-nesting geese**; however, no DNA was detected in tissues. We suggest the use of magnetic capture followed by quantitative real-time PCR (rather than a conventional PCR) to increase detection probabilities. Concerning a commercial MAT kit used on goose samples in the field, the use of fresh samples gave the best results in comparison with stored samples subjected to freezing and thawing. I demonstrated the potential for use of this commercial MAT kit in remote field conditions and communities in order to help screen potentially contaminated wild game meat, perhaps by training community monitors to test meat juice before consumption of undercooked or raw meat. A similar approach has been established in Nunavik regarding infections caused by *Trichinella nativa* related to walrus-meat consumption. By establishing a prevention program, they successfully eradicated outbreaks of trichinellosis in this region (Larrat et al., 2012). Implementing such programs in northern communities could however bring some challenges. Good communication and coordination to prevent negative effects on food security, involvement of the communities, training and education, as well as funding are aspects that must all be taken in account.

Finally, I successfully established subclinical infection in experimentally infected **reindeer**. No major detrimental effects were noted, and magnetic capture followed by conventional PCR demonstrated presence of the parasite in all tissue tested, including muscles commonly consumed by northern people. These findings emphasize the need to perform future work on *T. gondii* in naturally infected caribou regarding food-borne transmission.

Future work stemming from my thesis could include exploring vertical transmission in goose eggs as well as isolation and characterization of *T. gondii* in Arctic foxes and geese in the Karrak Lake region in order to better understand how the parasite thrives in this ecosystem and to determine its origin and the extent of genetic diversity in the Arctic. A few studies have looked into eggs of experimentally infected chicken and ducks but they found no clear evidence of *Toxoplasma* transmission (Biancifiori et al., 1986; Bartova et al., 2004; Dubey, 2010a). Embryonic mortality in eggs of experimentally infected chicken has

however been noted (Biancifiori et al., 1986). One older study claimed to have found *T. gondii* in eggs of experimentally infected broiler chicken (Kinjo, 1961); however, it seems to be very uncommon (Dubey, 2010a). Finally, ongoing study of prevalence of *T. gondii* antibodies in this fox population would be necessary to assess any fluctuation of serostatus over the years and determining if any problems are correlated with this parasite at an individual and population level. With warmer temperatures and anthropogenic factors changing the ecological balance and context within which disease hosts or vectors and parasites breed, develop, and transmit disease (Patz et al., 2000), transmission of *T. gondii* across circumpolar ranges is more likely to increase in a near future. An augmentation of precipitation, changes in ecology of transport hosts like arthropods or rodents, as well as an increase of urbanization in the North are all variables that could profit *T. gondii*. Sporulated oocysts can remain viable for a number of years in moist environments and are known to be extremely resistant to many environmental conditions (Lindsay et al., 2002). With mean winter temperature increases, oocyst survival is more likely to increase (Meerburg and Kijlstra, 2009).

Wildlife of conservation concern has been threatened by *T. gondii*: for example, Australian marsupials. Being highly susceptible hosts for this parasite, *T. gondii* can cause both chronic and acutely fatal infection in these species (Parameswaran et al., 2009), as well as in endangered birds (Work et al., 2000). Sea otters in California have also been suffering from toxoplasmosis, contributing largely to the slow rate of population recovery (Conrad et al., 2005). Therefore, it is crucial to keep monitoring Arctic wildlife for *T. gondii* in order to assess any changes in its distribution, and if it causes any harm to threatened wildlife as well as people living in the High Arctic. The fragile balance characterizing animal, people and the environment in the circumpolar North highlights the necessity to pursue research towards zoonoses like *Toxoplasma gondii* in a rapidly changing Arctic in an effort to preserve Northern ecosystems and cultures for generations to come.

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